STABILITY STUDIES ON CERTAIN PARENTERAL CIPROFLOXACIN ADMIXTURES USING STABILITY INDICATING ASSAY

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The Tamil Nadu Dr. M.G.R. Medical University, Chennai

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Submitted by

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CERTIFICATE

This is to certify that the research work entitled **"STABILITY STUDIES ON CERTAIN PARENTERAL CIPROFLOXACIN ADMIXTURES USING STABILITY INDICATING ASSAY"** was carried out by **Vishnupriya.R** in the Department of Pharmaceutics, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, which is affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, under my direct supervision and guidance to my fullest satisfaction.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

PD	-	Peritoneal Dialysis
UV	-	Ultra Violet
I.V	-	Intra Venous
RP-HPLC	-	Reverse Phase High Performance Liquid Chromatography
HPTLC	-	High Performance Thin Layer Chromatography
MIC	-	Minimum Inhibitory Concentration
NCCLVP	-	National Coordinating Committee on Large Volume Parenterals
CI	-	Continuous Infusion
CCPD	-	Continuous Cycling Peritoneal Dialysis
ICH	-	International Conference on Hormonization
NCCLS	-	National Committee for Clinical Laboratory Standards

PURPOSE OF STUDY

Stability of a pharmaceutical product may be defined as the capability of a particular formulation, in a specific container/closure system, to remain within its physical, chemical, microbiological, therapeutic and toxicological specifications. Drug stability and compatibility are critical issues controlling accurate and appropriate delivery of drug therapy to patients. Stability is very important for antibacterial agents especially those given by I .V route as they reach systemic circulation directly, and the clinical outcome and safety are directly correlated to drug levels in blood.

Ciprofloxacin and Metronidazole are extensively used intravenous antimicrobial agents. The parenteral admixture of ciprofloxacin with metronidazole is considered as a valuable tool for antibacterial therapy when combined aerobic and anaerobic infections are involved (E. Vega et al. 2001).

Peritonitis remains a significant cause of morbidity and mortality in Peritoneal Dialysis (PD) patients who need parenteral antibiotics to be given along with the PD solution. Ciprofloxacin is one of the important antibacterial agents used in peritonitis (Mawhinney WM et.al.1992).

Most monograph literature on ciprofloxacin and metronidazole indicate stability of the two drugs at room temperature (25º C) and refrigeration (5º C). But in a temperate country like India, temperature reaches up to 50º C in summer. Hence we were interested in studying the stability of ciprofloxacin-metronidazole I.V admixture and the stability of ciprofloxacin in peritoneal dialysis solution at higher temperature (45ºC) by stability indicating assay method.

In the case of ciprofloxacin-metronidazole I.V admixture, both the drugs were monitored simultaneously using a stability indicating first derivative spectrophotometric method and the I.V admixture could be used successfully in

clinical therapy of mixed aerobic-anaerobic infections if it was proved to be stable. Similarly, stability of ciprofloxacin in peritoneal dialysis solution was studied using stability indicating HPTLC method, in addition to a microbiological assay which is a direct measure of the biological activity of antimicrobial agents.

ABSTRACT OF WORK DONE

The physical and chemical stability of Ciprofloxacin I.V- Metronidazole I.V and Ciprofloxacin-Metronidazole I.V admixture were individually determined at 45°C, 25°C and 5°C. Ciprofloxacin and Metronidazole were quantified by using a stability indicating first derivative UV spectrophotometric method (E.Vega et.al; 2001

The physical and chemical stability of Ciprofloxacin when admixed with Peritoneal Dialysis (PD) solution was determined at 45°C, 25°C and 5°C and quantified by using a stability indicating HPTLC method (Jan Krzek et.al; 2005) in addition to a microbiological assay using *E.coli* – NCIM 2911 as test organism.

Decrease in drug concentration by more than 10% from initial concentration (0 time) was considered unstable (chemical instability). Change in pH by more than 1 unit was considered unstable (physical instability) (Narine Baririan et.al, 2003).

The drug solution was clear and colorless, but the pH decreased with time, though not to the extent of being considered physically unstable.

First derivative UV spectrophotometric analysis indicated that 2mg/ml concentration of Ciprofloxacin I.V when tested alone maintained adequate stability for 4 hours at 45°C, for 24 hours at 25°C and up to120 hours at 5°C. 5mg/ml concentration of Metronidazole alone maintained adequate stability for 6 hours at 45°C, for 24 hours at 25°C and up to 120 hours at 5°C and the I.V admixture of ciprofloxacin with metronidazole (1:2.5) was stable for less than 4 hours at 45°C, for 24 hours at 25°C and up to 120 hours at 5°C.

HPTLC analysis indicated that 400µg/ml concentration of ciprofloxacin I.V in peritoneal dialysis solution maintained adequate stability for 4 hours at 45°C, for 24 hours at 25°C and up to 120 hours at 5°C. As per microbiological assay, Ciprofloxacin in peritoneal dialysis solution was stable up to the study period of 6 hours at all the three temperatures.

INTRODUCTION

Stability of a pharmaceutical product may be defined as the capability of a particular formulation, in a specific container, to remain within its physical, chemical, microbiological, therapeutic and toxicological specification (USP 22).

USP (22) defines stability as the extent to which products retains within specified limits and throughout its period of storage and use i.e. its shelf life, the same properties and characteristics that it possessed at the time of manufacture.

Stability of a drug can also be defined as the time from the date of manufacture and packaging of the formulation until its chemical or biological activity is not less than a pre-determined level of labeled potency and its physical characteristics have not changed appreciably or deleteriously.

REASONS BEHIND STABILITY STUDY: (Pulok. K.Mukherjee. et.al., 1994)

Pharmacists have to play a vital role in respect of stability of the pharmaceuticals as:

a) For the safety of the patient, it is important to note that the patient must have to receive an uniform dose of drug throughout the shelf life of the product. Although a drug may have been shown to be safe for use, this is not necessarily true of the decomposed products. Manufacturers must have to minimize or prevent decomposition of products.

b) Consideration must be given to the relevant legal requirements concerned with identity, strength, purity and quality of the drug.

c) It is important to prevent the economic repercussions of marketing an unstable product.

d) In any rational design and evaluation of dosage form, the stability of the active component must be a major criterion in determining acceptance or rejection of trial formulations. Several forms of instability that can lead to rejection of a drug product are:

- With the advancement of medicinal chemistry, drugs being used are highly specific, chemically complex, and potent and most of them have a very narrow therapeutic range. If there is an extensive chemical degradation of the active drug, it leads to a substantial lowering of the quantity of the therapeutic agent in the dosage form. In these cases it is of paramount responsibility of the pharmacists, to see that the drug dosage form can reproducibly deliver the same amount of drug.
- A very toxic product may be formed in the decomposition process. There the decomposed products are more toxic than the original therapeutic agent, which when ingested, can cause undesirable effects. (E.g.) p-amino-salicylic acid to m-aminophenol.
- Instability of a drug product may lead to decrease in bioavailability. This reduction in bioavailability can lead to a substantial lowering in the therapeutic efficacy of the dosage form. This phenomenon may be due to physical and chemical changes of the diluents in the dosage form, independent of whatever changes the active drug may have undergone.
- There may be substantial changes in the physical appearance of the dosage form. This may not alter the therapeutic efficacy of the dosage form but most likely may result in loss of confidence of the patient in the drug product, which then has to be rejected.

Incompatibility (Levit J Demorest. et.al, 1986)

The phenomenon of incompatibility occurs when the LVP (large volume parenteral) and drug produce, by physicochemical means, a product that is unsuitable for administration to the patient. Physical incompatibility may be detected by a change in the appearance of the solution, such as the formation of a precipitate, a haze, a change of color, or the breaking of an emulsion, Subtle incompatibilities, such as a change in pH or drug concentration, may not result in a visual change or may not become evident until a later time.

Instability occurs when an LVP product or admixture is modified due to sorption or such storage conditions as time, light, or temperature. The modified product may not be suitable for administration, and unless the combination has been studied in the laboratory, the only clue to a stability problem may be failure to get the expected clinical result.

The parameters of tonicity, pH, solubility, and added substances, which were considerations in the design of the LVP formulation, also must be considered in a different context when drugs are added to the solution. The drug product may contain solvents, preservatives, stabilizers, buffers, and other ingredients that, when added in the LVP can result in instability and compatibility problem. Sodium benzoate, a preservative in some drugs precipitates as benzoic acid when added to an LVP with an acidic pH. Copper a trace metal needed by the body. Can cause precipitation in amino acid solution. The pharmacist must be knowledgeable of the ingredients in the LVP and in the drug product and possible incompatibilities when preparing admixtures.

Stability of the combination must be maintained after mixing and during infusion if the desired result is to be achieved. Stability problems may be caused by pH, solubility, sensitivity to light or temperature, absorption, or chemical incompatibility. Stability may also be related to time, and this is one reason that is recommended that admixtures not be stored for prolonged periods.

One example of the role of pH would be that be that of ampicillin B in dextrose solutions. Unless the pH of the dextrose solution is greater than 5.0, the combination is incompatible. The monograph for Dextrose for injection allows a pH range of 3.5 – 6.5. When the pH of 5% Dextrose in Lactated Ringer's injection is below 5, some nerve blocking agents, such as succinylcholine, will precipitate from solution.

Chemotherapeutic drugs and vitamin preparations generally should be protected from light.

Sodium bisulfite, an ingredient added to some LVP to reduce degradation caused by oxidation, may be present in only the quantity needed for protection of the solution during sterilization and shelf life. It may not be present in sufficient quantity to provide protection from the air that may be introduced to the container during admixing or storage in plastic containers.

The order of introduction of drugs to the LVP may either highlight or mask visible incompatibilities. If a drug is incompatible at a give pH and the pH of the LVP must be adjusted, the pH should be adjusted before the drug is added. A fat emulsion, white and opaque, masks reaction that might be visible in a clear solution and the package insert cautions not to add electrolytes directly to the emulsion.

Types of Incompatibilities:

Incompatibilities can be divided into three categories: therapeutic (pharmacologic), physical and chemical. “Therapeutic incompatibilities” occur when two or more drugs administered concurrently result in undesirable antagonistic or synergistic pharmacologic action. Examples of incompatibilities are the antagonism between chloramphenicol and penicillin and the possibility that penicillin or cortisone may antagonize the effect of heparin and produce a misleading picture of the anticoagulant effect of heparin.

When the combination of two or more drugs in solution results in a change in the appearance of the solution, such as a change in colour, formation of turbidity or precipitate, or the evolution of a gas, it is called a “physical incompatibility”. Physical incompatibilities are the easiest to detect and represent the types most familiar to hospital pharmacists. They can frequently be predicted from the chemical characteristics of the drugs involved. For example, the sodium salts of weak acids such as sodium diphenylhydantoin or sodium phenobarbital precipitate as free acids when added to intravenous fluids having an acidic pH. Available compatibility charts attempt to outline this type of incompatibility.

Degradation of drugs in solution resulting from the combination of parenteral dosage forms is called “Chemical incompatibility”. This is an arbitrary classification because physical incompatibilities also result from chemical changes. Most chemical incompatibilities result from hydrolysis, oxidation, reduction or complexation and can be detected only with suitable analytical methods.

Reasons for Incompatibility (Salvatore J. Turco, 2000):

Probably the greatest single factor in causing an incompatibility is a change in acid – base environment. The solubility and stability of a drug may vary as the pH of solution varies. A change in the pH may be a clue in predicting an incompatibility, especially one involving drug stability, since it is not necessarily apparent physically.

Penicillin provides a good example of the effect of pH on drug stability. The antibiotic in solution remains active for 24 hours at pH 6.5, but at pH 3.4 is destroyed in a short time. Potassium penicillin G contains a citrate buffer; therefore, the injection is buffered at pH 6.0 to 6.5 when reconstituted with sterile water for injection, 5% D/W, or sodium chloride injection. When this reconstituted solution is added to an intravenous fluid such as dextrose Injection of sodium chloride injection, the normal acid pH of the solutions is buffered at pH 6.0 to 6.5, thus ensuring the anitbiotic’s activity. Carbenicillin should not be admixed in the same solution as carbenicillin, Adding other drugs, such as metaraminol bitartrate, ascorbic acid, or tetracycline hydrochloride, that have a low pH to the intravenous fluid containing the penicillin may lower the pH to a point at which the penicillin may be inactivated. The same can happen if the reconstituted penicillin solution is added to intravenous fluids of high buffering capacity that have a pH below 6.0 such as Lactated Ringer’s Injection or protein hydrolysates.

In a similar manner, the addition of the reconstituted penicillin to solutions in the alkaline range can be deleterious. Dilute sodium bicarbonate solutions are sometimes added to 5% distilled water to reduce the incidence of phlebitis, or the bicarbonate ion may be used for treating acidosis. Studies have shown that penicillin present in solution at pH 8.5 retain only 25% of its activity after 6 hours and 1% after 24 hours. With higher temperatures or higher pH, the inactivation of the antibiotic is accelerated.

The stability of the antibiotic sodium ampicillin is also pH dependent. When reconstituted sodium ampicillin is added to 5% distilled water at the concentration of 30 mg / ml, its period of stability under the prevalent acid conditions is 4 hours. When added to sodium chloride injection, however its loss in activity is less that 10% in 8 hours. For this reason sodium chloride injection is the intravenous fluid for sodium ampicillin.

Drug stability and compatibility issues in drug delivery (Lawrence A. Trissel, 1998):

Drug stability and compatibility are critical elements in the accurate and appropriate delivery of the drug therapy to patients. Both the therapeutic adequacy of the treatment and the safety and the therapy can be adversely affected by drug instability or drug incompatibility.

The term instability is usually applied to chemical reactions that are incessant, irreversible and result, in distinctly different chemical entities (degradation products) that can be both therapeutically inactive and possibly exhibit greater toxicity.

Incompatibility generally refers to physiochemical phenomenon such as concentration – dependant precipitation and acid base reactions with the product of reaction manifested as a change in physical state or protonation – deprotonation equilibrium. When the incompatibility results in visible change(s) in color or viscosity, effervescence or the formation of immiscible liquid layer the term physical incompatibility or more accurately visual incompatibility is used.

Expiration time shelf life (or) utility time restrictions result from the drug instability or incompatibility as well as from other factors such as maintenance of sterility. Typically these terms indicate the period for which a minimum of 90% of the drug remains intact and available for delivery. Most often, an expiration date is applied to the indirect formulation from the manufacture, while a much shorter utility time is determined for the drug when constituted and diluted for the administration. The term shelf life has been applied to both of these situations.

When formulating a drug into a suitable parenteral dosage form, a manufacturer uses adjuvants or added substances to obtain a stable solution. When a drug is added to an intravenous fluid, the conditions of the drug are changed. Drugs packed under an inert atmosphere such as nitrogen, because of their ease of oxidation, loose this protection and the value of any antioxidant present when they are added to the intravenous fluid. The added substances in one injection may not be compatible with the drug and the added substances in another when they are placed together in intravenous fluid. Placing two injections together in the same syringe may accentuate the problems because of the concentrations involved and the difficulty in thoroughly mixing the injections, creating what is called a “layering effect”.

Many injections have special storage requirements such as protection from light or refrigeration. Using these solution with intravenous fluids, not administering them immediately, or administering them for prolonged periods may produce conditions not favorable to the drugs stability.

The primary IV fluid is usually a carbohydrate, electrolyte, or amino acid solution. Carbohydrate solutions are not usually a problem with additives; although they are acidic, they have negligible buffer capacity, and additive determines the pH. Calcium and phosphate in TN (Total Nutrition) solution require special care in mixing. Lactate, acetate and gluconate present as salts can act as buffer and resist changes in pH that may make the additive insoluble. Amino acid–containing IV solutions may degrade acid-labile drugs, bind drugs or

form complexes. No drug additives should be mixed with fat emulsion (Intralipid).

Drug products must meet stability standards for long-term storage at room temperature under the condition of relative humidity. Drug instability in pharmaceutical formulations may be detected in some instances by a change in the physical appearance, color, taste, odor or texture of the formulation were as in other instances chemical changes may occur which are not self-evident and may not be ascertained through chemical analysis.

The study of the rate of the chemical change and the way in which it is influenced by such factors as the concentration of the drug are reacted, the solvent employed, the conditions of temperature and pressure, and the presence of other chemical changes in the formulations is termed reaction kinetics.

In general a kinetic study begins by measuring the concentration of the drug being examined at given time intervals under a specific set of conditions including temperature, pH, ionic strength, light intensity and drug concentration. The measurement of the drug concentration at various time intervals reveals the stability or instability of the drug under the specified conditions with the passage of time. From this starting point, each of the original conditions may be varied on an individual basis to determine the influence that such changes make on the drug's stability. For e.g. The pH of the solution may be changed, whereas the temperature, light intensity, and original drug concentration remains as they were in the original or baseline experiment.

In addition to the accelerated stability, drug products are also subjected to long-term stability studies under the usual conditions of transport and storage expected during product distribution. In conducting these studies, the different climatic zones, nationally and internationally, to which the product may be subjected must be borne in mind and expected variances in conditions of temperature and humidity included in the study design.

When chemical degradation products are detected, the FDA (Food and Drug Administration) requires the manufacturer to report their chemical identities, including structure, mechanism of formation, physical and chemical properties, procedures for isolation and purification, specifications and directions for determining at levels expected to be present in the pharmaceutical products, and the pharmacological action and biological significance, if any, to their presence.

Stability of Parental products (James C Boylan. et.al, 1995):

A formal stability program is needed to assure that all critical attributes of any drug product are maintained through out the shelf life of the product. A validated stability-testing assay is essential to measure chemical or biological activity, and acceptance criteria

Should be established before initiating stability studies. Particular attention should be given for developing a detailed protocol for a stability study before preparing stability samples, including assays to be performed, storage conditions and sampling intervals

In general, expiration dating is based on the estimated time required for the active compound to reach Labeled potency at the specified room temperature. The drug substance itself may be subjected to physical instability such as adsorption. The stability program should include placing enough units at the specified storage conditions to allow inspection of a statistically valid number of units to verify acceptable appearance of the product, such as the development of haze and or discrete particulate matter in solution products, as well as to check for discoloration or any other physical attribute that would result in un acceptable pharmaceutical elegance. Formulation pH is often a critical attribute that must be monitored during a stability study, since pH may be affected by both by chemical reactions or by interactions between the formulation and the container closure system

Sterile powder may require special attention to identify which tests are required to assure adequate physical and chemical stability. The stability of many dried products is often sensitive to small amount of residual water present, requiring monitoring of residual moisture by Karl Fischer titration or loss on drying. This is particularly important for protein formulations.

For freeze dried products cake shrinkage with time is uncommon. This may be accompanied with discoloration, increased reconstitution time, or crystallization of one or more compounds of the formulation the physical state other drugs-crystalline or amorphous-has an important influence on stability, particularly for cephalosporin's

Parenteral Dosage Forms And Their Stability Profiles :

Signs of degradations of the specific dosage forms must be observed and reported. For some of the dosage forms, this includes the following:

Small Volume Parenterals :

Strength, appearance, color, particulate matter, dispersibility (suspension), pH, sterility, pyrogenicity and closure integrity.

Large Volume Parenterals :

Strength, appearance, color, particulate matter, pH, volume and extractable (when plastic containers are used), sterility, pyrogenicity and closure integrity.

Parenteral Admixtures :

The combination of parenteral dosage forms for administration as a single entity is called “admixture”. Because of convenience, intravenous fluids are frequently used as vehicles, or carriers, for other parenteral drugs. The drug placed in solution in another parenteral dosage is the additive.

Numerous studies have been done to determine the extent of admixture use. Studies at the National Institute of Health (NIH) and the University of Michingan showed that 50 to 70% of the intravenous fluids administered contain additives. Other survey showed that the majority of the orders received contained 1 or 2 additives, and a small percentage required as many as 6 additives to be administered in the same intravenous fluid.

Theoretically, the number of possible combination of parenteral drugs in intravenous fluids is staggering. A group at the University of Chicago calculated that 24 drugs added in groups of 2 to 5% distilled water produced 276 unique pairs. In this particular hospital, there was the possibility of using any one of 36 different intravenous fluids as the vehicle thus brining the number of possibilities to over 9000. If the 24 drugs were used in combinations of twos, threes and fours when added to 5% distilled water, these combinations produced 11,000 unique admixtures. When varied with the 36 different intravenous solutions these 24 drugs could produce over 396,000 possible combinations. The inclusion of any of the other hundreds of parenteral drugs available with the 24 used in this study rapidly increases the number of possibilities. Recognizing the extent of possible combinations is important in seeking a reasonable and practical solution to a problem.

Other important factors in considering the number of combinations possible include brand variations, drug concentration, added substances, order of mixing, and time elapsing prior to administration.

As the number of additives increased, the likelihood of creating conditions unfavorable for the stability of one or more of them also increases. Aminophylline Injection added to Sodium chloride injection already containing vitamin B complex and vitamin C as additives results in an alkaline solution unfavorable to the stability of the vitamins.

Complexation can occur between drugs, rendering them inactive. Tetracycline in the presence of calcium ions forms a complex that reduces the tetracycline activity.

Parenteral Incompatibility:

The physical, chemical and therapeutic problems that arise when parenteral drugs are combined administered by injection are called “parenteral incompatibility”. The tendency of practitioners to prescribe several drugs combined and administered together is prompted by the convenience and time saving features of the practice, the reduction in the number of injection’s necessary, an attempt to treat several conditions simultaneously, and the ability to give the drugs in controlled increments. Although problems have probably existed since the acceptance of the parenteral method of administration, only recently have they become a concern to practitioners, owing to the number of increasing use of large volume parenteral fluids as vehicles for other drugs.

Large –volume fluids have their own therapeutic uses; they were not necessarily formulated to act as carrier’s or vehicle’s for other drugs. Likewise, parenteral drugs used as additives were not necessarily formulated to remain stable and active when added to one of a variety of large – volume fluids. Nevertheless, current medical practice results in this use for these solutions and the potential problem of parenteral incompatibility arises.

The NCCLVP (National Coordinating Committee on Large volume Parenterals) has defined incompatibility as a phenomenon that occurs when one drug is mixed with others to produce, by physiochemical means a product

unsuitable for administration to the patient. The patient may not receive the full therapeutic effect of the mixture, or toxic decomposition products may result. The precipitated incompatibility may irritate the veins or cause occlusion of vessels.

Many hospitals have established pharmacy intravenous additive programs, which have centralized the responsibility, increased the uniformity, and improved the safety of combining drugs for parenteral administration. Such services have been a contributing factor in improving patient care.

Intravenous fluids:

Large volume injections intended to be administer by intravenous infusion commonly are called I.V fluids and are included in the group of sterile products referred to as large volume parenterals. These consist of single dose injections having a volume of 100mlor more and containing no added substances. Mini type infusion containers of 250ml capacity are available with 50ml and 100ml partial fills for solution of drugs used in the piggy bag technique. In addition to the IV fluids, this group also includes irrigation solutions and solution for dialysis.

Intravenous solutions are sterile solutions of simple chemicals such as sugars, amino acids, or electrolytes-materials that easily can be carried by the circulatory system and assimilated. Prepared with water for injection USP, the solutions are pyrogen free, because of the large volumes administered intravenously, the absence of particulate matter assumes a significant role in view of possible biological hazards resulting from insoluble particles, absence of particulate matter or clarity of IV fluids

Is as important at the time of administration following their manipulation in the hospitals as it is at the manufacture of injection.

Limits of particulate matter occurring in IV fluids or large volume injections used for single dose infusion are defined in the USP. Limits also apply to multiple dose injections, mall volume injections prepared by reconstitution

from sterile solids. The USP defines particulate matter as extraneous, mobile, undissolved substances other than gas bubbles, unintentionally present in parenteral solutions.

Intravenous fluids are commonly used for a serious of body condition. These include

- Correction of disturbances in electrolyte balance
- Correction of disturbances in body fluids
- The means of providing basic nutrition
- The basis for the practice of providing parenteral nutrition
- Vehicles for other drug substances

In both of the latter two cases it has become common practice to add other drugs to certain IV fluids to meet the clinical needs of the patient. Using IV fluids as vehicles offers the advantage of convenience, the means of reducing the irritation potential of the drug, and a method for continuous drug therapy. However the practice requires that careful considerations be given to the stability and compatibility of additives present in the IV fluids serving as the vehicle. This approach also demands strict adherence to aseptic techniques in adding the drug as well as in the administration of the IV fluids.

Intravenous admixture (Salvatore J Turco. 2000) :

It has been estimated that 40% of all drugs administered in hospitals are given in the form of injections, and their use is increasing. Part of this increase in parenteral therapy is due to the wider use of intravenous fluids (I.V fluids).

Not only to I.V fluids continue as the means of fluid replacement, electrolyte balance restoration, and supplementary nutrition, they also are playing major roles as vehicles for administration of other drug substances and in total parenteral nutrition

The parenteral prescription is becoming increasingly important in hospitals. Centralized admixture program are found in 90%of the Nation's hospitals with 300 beds or more in USA. Equipment available for administering I.V fluids has become sophisticated and has made possible increased accuracy of dosage and led to the development of new concepts and methods of nutrition and drug therapy.

New methods of IV drug delivery systems have been introduced and are constantly evolving. The introduction of patient controlled analgesia is commonplace in hospitals. This technology allows the patient with pain to control the degree of analgesia.

Packaging of parenteral in the past 5 years also has undergone dramatic changes. The manufacturers now supply prefilled, prefrozen, premixed parenterals. The pharmaceutical industry has responded to the needs of pharmacists by addressing the packaging, labeling, and design requirements necessary to facilitate patient care.

Minimization Of Incompatibilities (Lawrence A . Trissel, 1998):

Although it may be impossible to predict and prevent all parenteral incompatibilities, practicing the following general principles is helpful in minimizing the difficulties.

- Uses freshly prepare solutions if possible; periodically observing the running of the intravenous fluid to detect changes in appearance. Discard any unused solution after 24 hours. Refrigeration may be requiring if it is necessary to prepare admixture in advance. Proper storage temperatures must be maintained prior to infusion. Although furosemide (Lasix) does not require refrigeration, it is sometimes stored under refrigeration in prefilled syringes to reduce the effects of light. This can cause crystallization and should not be done. Cephaloridine (Loridine) injections become milky white during refrigeration and should be returned to room temperature prior to use.

- Encourage the use of as few additives as possible in infusion fluids. As additives increase, so the number of potential problems. When one concludes that vitamin B complex with vitamin C may contain up to 10 drug substances in addition to the pharmaceutical adjuvant present, the potential problems occurring becomes clear. Mixing thoroughly after each additive addition prevents concentrated layering effects.

- Dilution may prevent incompatibility; for e.g.: erythromycin lactobionate is incompatible with electrolytes in concentrated form; however; it is compatible when properly diluted. It will form a solid gel in a small vial of 0.9% sodium chloride; in a liter bottle of 0.9% sodium chloride, it will dissolve completely.

- Become knowledgeable about parenteral therapy. The practitioner can become aware of incompatibilities through the literature.

- Make physicians and others aware of possible incompatibilities and always be ready to suggest alternate approaches to avoid the difficulties. In some instance, compatibilities can be avoided by selecting another route or site of administration for one of the drugs involved.

- The preparation of the admixture should be accomplished using aseptic techniques in a proper area. The containers of the injections to be used should be examined for acceptability in all aspects, such as clarity, absence of cracks in the glass, and presence of vacuum if the system requires it.

- Keep a file of available data and add to it from your own experience and literature.

- Utilize available compatibility charts. Although these charts have limitations, the proper use of the information can be helpful in predicting incompatibilities. Most charts list only physical incompatibilities, they cannot be used for chemical or therapeutic incompatibilities. The chart for other

systems can be helpful, but they cannot be depended on categorically to rule out a problem. The same injections prepared by different companies may differ in pH values. If the brand or source of additive varies from the one used in compilation of the chart, the results may differ because different substances are used by various companies to prepare the additives,. If the concentration of the drug in a specific problem is different from that used in compiling the chart the results may also be different. The data in the chart are concentration–dependent and the concentration per unit volume is frequently specified. Charts are updated form time to time; thus the age of the data can influence results.

If the order of mixing is different from the order used in preparing the chart, the results can be different. Most charts specify a definite period of observation, usually 24 hour

- It is impossible to chart or discuss every possible admixture. Instead, principles must be learned and applied; for e.g. no drug should ever be added to blood or blood products. According to the charts almost every type additive causes an incompatibility when added to protein hydrolysate solutions. Yet it is common practice to combine several additives in hyperalimentation solutions. Penicillin form potentially allergenic conjugates with proteinaceous materials. Therefore it is not advisable to add them to protein hydrolysate solution. Other general principles to remember are that penicillins are inactivated at either low or high pH values; sympathomimetic amines are specially sensitive when added to intravenous fluids; and therapeutic large–volume solutions such as sodium bicarbonate, urea, mannitol, dextran, and L-arginine should never contain additives.

ICH GUIDELINES ON STABILITY: (ICH guidelines, 1993) :

One of the constants in our industry is the continuing need to develop information about the stability of active pharmaceutical ingredients and drug products we work with. The need to know the stability of materials that are in development, that are in the clinic and that are being manufactured commercially has resulted in a number of regulatory documents describing the procedure that should be used to generate the required stability information.

The International Conference on Harmonization (ICH) has published extensively on the subject of stability. As a part of the ICH, FDA has adopted the ICH guidelines on the conduct of stability studies.

The first ICH guideline on stability reached step 4 of the ICH process and was recommended for adoption in October 1993. The guideline was revised in 1999 and the revised guideline was recommended for adoption in November 2000.

These Guidelines are:

- Stability Testing: photo stability testing of new drug substances and products Q1B (1996)
- Stability Testing for New dosage forms: Annex to the ICH Tripartite guideline on stability testing for new drugs and products Q1C (1996)
- Bracketing and matrixing designs for stability testing of new drug substances and products Q1D (2002)
- Evaluation for stability data Q1E (2003)
- Stability data package for registration applications in climatic zones 3 and 4 Q1F (2003)

The purpose of a stability study is to establish, based on testing a minimum of three batches of the drug substance or product, a retest period or

shelf life and label storage instructions, applicable to all future batches manufactured and packaged under similar circumstances.

Guideline Q1A and the first revision established the general case for stability studies as:

Storage condition	Temperature	Relative humidity
Long term storage condition	25ºc ±2ºc	60% RH ±5% RH
Intermediate Storage condition	30ºc ± 2ºc	65% RH ±5% RH
Accelerated storage condition	40ºc ± 2ºc	75% RH ±5% RH

The intermediate storage condition has been changed to:

30ºc ± 2ºc/ 65% RH ±5% RH for drug substances and drug products. The stability of a product depends not only on the nature of the packaging material but also on factors such as conditions during packaging, pack design and pack geometry, including head space. Over protective packaging adds to the cost of packaging; therefore it is important to determine what packaging is really necessary in each case..

DRUG PRODUCT

General

The design of the stability programme for the finished product should be based on the knowledge of the behavior and properties of the drug substance and the experience gained from clinical formulation studies and from the stability studies on the drug substance. The likely changes on storage and the rationale for the selection of product variables to include in the testing programme should be stated.

Selection of Batches

Stability information from accelerated and long term testing is to be provided on three batches of the same formulation and dosage form in the containers and closure proposed for marketing. Two of the three batches should be at least pilot scale. The third batch may be smaller (e.g., 25,000 to 50, 0000 tablets or capsules for solid oral dosage forms). The long term testing should cover at least 12 months duration at the time of submission. The manufacturing process to be used should meaningfully simulate that which would be applied to large-scale batches for marketing. The process should provide product of the same quality intended for marketing, and meeting the same quality specification as to be applied for release of material.

Data on laboratory scale batches is not acceptable as primary stability information. Data on associated formulations or packaging may be submitted as supportive information. The first three production batches manufactured post approval, if not submitted in the original registration application, should be placed on accelerated and long term stability studies using the same stability protocols as in the approved drug application.

Test Procedures and Test Criteria

The testing should cover those features susceptible to change during storage and likely to influence quality, safety and efficacy. Analytical test procedures should be fully validated and the assays should be stability indicating. The need for the extent of replication will depend on the results of validation studies.

The range of testing should cover not only chemical and biological stability but also loss of preservative, physical properties and characteristics, organoleptic properties and where required, microbiological attributes. Preservative efficacy testing and assays on stored samples should be carried out to determine the content and efficacy of antimicrobial preservatives.

Specifications

Limits of acceptance should relate to the release limits (where applicable), to be derived from consideration of all the available stability information. The shelf life specification could allow acceptable and justifiable derivations from the release specification based on the stability evaluation and the changes observed on storage. It will need to include specific upper limits for degradation products, the justification for which should be influenced by the levels observed in material used in pre-clinical studies and clinical trials.

Storage Test Conditions

The length of the studies and the storage conditions should be sufficient to cover storage, shipment and subsequent use (e.g., reconstitution or dilution as recommended in the labeling). An assurance that long term testing will continue to cover the expected shelf life should be provided.

Other storage conditions are allowable if justified. Heat sensitive drug products should be stored under an alternative low temperature condition, which will eventually become the designated long-term storage temperature. Special consideration may need to be given to products, which change physically or even chemically at lower storage conditions. Where a lower temperature condition is used, the six months accelerated testing should be carried out at a temperature at least 15ºC above its designated long term storage temperature (together with appropriate relative humidity conditions for that temperature). For example for a product to be stored long term under refrigerated conditions, accelerated testing should be conducted at 25ºC ± 2ºC / 60 percent RH ± 5 percent RH. The designated long term testing conditions will be reflected in the labeling and expiration date.

Storage under conditions of high relative humidity applies particularly to solid dosage forms. For products such as solutions, suspensions etc., contained in packs designed to provide a permanent barrier to water loss, specific storage under conditions of high relative humidity is not necessary but the same range of

temperatures should be applied. Low relative humidity (e.g. 10-20 percent RH) can adversely affect products packed in semi-permeable containers (e.g. solutions in plastic bags, nose drops in small plastic containers etc) and consideration should be given to appropriate testing under such conditions.

Conditions	**Minimum time period at submission**
Long term testing 25ºC ± 2ºC / 60% RH ± 5%	12 Months
Accelerated testing 40ºC ± 2ºC / 75% RH ± 5%	6 Months

Where significant change occurs due to accelerated testing, additional testing at an intermediate condition e.g.

30ºC ± 2ºC / 60% RH ± 5% RH should be conducted.

Significant change at the accelerated condition is defined as:

1. A 5 percent potency loss from the initial value of a batch.
2. Any specified degradant exceeding its specification limit.
3. The product exceeding its pH limits.
4. Dissolution exceeding the specification limits for 12 capsules or tablets.
5. Failure to meet specifications for appearance and physical properties e.g., color, phase separation, resuspendibility, delivery per actuation, caking, hardness etc.

Should significant change occur at 40ºC/75 percent RH then the initial registration application should include a minimum of 6 months data from an ongoing one year study at 30ºC/60 percent RH; the same significant change criteria shall then apply.

The long term testing will be continued for a sufficient time beyond 12 months to cover shelf life at appropriate test periods.

Testing Frequency

Frequency of testing should be sufficient to establish the stability characteristics of the drug product. Testing will normally be every three months over the first year, every six months over the second year and then annually.

Evaluation

A systematic approach should be adopted in the presentation and evaluation of the stability information which should cover all necessary physical, chemical, biological, microbiological quality characteristics, including particular properties of the dosage form.

The design of the stability study is to establish, based on testing a minimum of three batches of the drug product, a shelf life and label storage instructions applicable to all future batches of the dosage from manufactured and packed under similar circumstances.

Where the data shows so little degradation and so little variability that it is apparent from looking at the data that the requested shelf life will be granted, it is normally unnecessary to go through the formal statistical analysis but only to provide a justification for the omission.

Any evaluation should consider not only the assay, but also the levels of degradation products and appropriate attributes. Where appropriate, attention should be paid to reviewing the adequacy of the mass balance, different stability and degradation performance.

Statements/Labeling

A storage temperature range may be used in accordance with relevant national/regional requirements. The range should be based on the stability evaluation of the drug product. Where applicable, specific requirements should be stated particularly for drug products that cannot tolerate freezing. There should

be a direct linkage between the label statement and the demonstrated stability characteristics of the drug product.

CLASSIFICATION OF STABILITY STUDIES:

Stability studies can be broadly classified as follows:

- Accelerated studies
- Long term studies

ACCELERATED STUDIES:

It is generally conducted on actual formulations for several weeks under accelerated conditions of temperature, humidity or light. The primary objective of these studies is to obtain an early reading on whether a given active ingredient or formulation is likely to be sufficiently stable to deserve detailed exploration.

In performing these studies, an active ingredient or a formulation can be subjected to much higher temperatures, humidity or light conditions than it is likely to be subjected to under normal transportation, storage and usage conditions. For example, an active ingredient may be subjected to increasing temperatures until decomposition is detected

LONG TERM STABILITY:

Formulations are designed to maintain or enhance the stability of the active ingredients while allowing optimal delivery in the human body. The design is based on the physicochemical properties of the active ingredient and its compatibility with the excipients. Many approaches are used to stabilize or protect formulations including lyophilization, microencapsulation, control of surface area, addition of chelating agents, preservatives, and antioxidants, physical separation of incompatible ingredients, coatings and opaque coverings.

The stabilization of a dosage form-container combination is extrinsic to the stability of the dosage form. The container is an integral part of products such as topical and parenteral.

STORAGE CONDITIONS:

An important part of stability evaluation is the determination of the effects of environmental conditions on the product. The factors commonly tested are heat, humidity, light and air. Data obtained from these studies form the basis for the establishment of an expiration date. Temperature can vary from one place to other therefore; it is best to relate laboratory storage of stability samples to actual market conditions.

Storage conditions stipulated in individual monographs are defined as:

- Cold - Any temperature not exceeding 8ºC.
- Cool - Any temperature between 8 and 15ºC.
- Room temperature – Between 15 and 30ºC.
- Warm - Any temperature between 30 and 40ºC.
- Excessive heat - Any temperature above 40ºC

The climatic conditions around the world can be classified into the following four categories:

I. Temperate climate (20ºC, 42% RH)
II. Subtropical climate (21.6ºC, 52% RH)
III. Hot, dry climate (30ºC, 40% RH)
IV. Hot, humid climate (30ºC, 70% RH)

There are three bases upon which pharmacists are able to recognize, predict and avoid instability in parenteral solutions (David. W. Newton, 1978) they are:

1. Professional judgment derived from direct experience.
2. A competent understanding of physicochemical principles with particular emphasis on common reactive and labile groups.

3. Reference to pertinent published information.

STABILITY INDICATING METHODS: (Jens T. Cartensen, 2005) :

A stability indicating method is an analytical procedure that is capable of discriminating between the major active (intact) pharmaceutical ingredients (API) from any degradation (decomposition) product(s) formed under defined storage conditions during the stability evaluation period.

FDA defines stability indicating methods as a quantitative analytical methods that are based on the characteristic structural, chemical or biological properties of each active ingredient of a drug product and that will distinguish each active ingredient from its degradation products so that the active ingredient content can be accurately measured.

Developing a stability indicating assay requires consideration of three aspects of the method:

1. Obtaining a representative sample
2. Choosing the separation technique and
3. Selecting the detector.

1. Obtaining a representative sample:

To obtain samples for developing stability indicating assays the pure drug substance were placed under stress intentionally. This process is often called forced degradation or purposeful degradation. The drug may be subjected to acid, base, heat, light or oxidation for stress degradation. Usually, the goal is to degrade the parent drug by 10-20% or so. Degradation much greater than 10-20% could result in secondary degradants that will complicate the development process.

2. Choosing the separation technique:

a. Chromatographic methods:

A large number of stability indicating methods entail some form of chromatography: thin-layer, gas (GLC), liquid (HPLC), or supercritical fluid (SFC). Of these methods HPLC has found the greatest application.

i. HPLC

In this method, the samples are generated in aqueous solutions. The polarity of the degraded samples can vary widely; for example, when a non-polar drug is degraded into smaller polar components, then gradient elution is employed for sample screening.

ii. HPTLC

In this method, the drug can be effectively separated from its degradation products. All the peaks of degradation products can be well resolved from the standard drug with significant difference in R(f) values.

b. Spectrophotometric method:

Derivative spectrophotometry presents greater selectivity than the normal technique and overcomes the problem of resolving spectral overlap in the analysis of a multicomponent system. A characteristic of this technique is that the differentiation discriminates against broad bands, emphasizing sharper features to an extent that they increase with increasing derivative order.

Selectivity can be improved through chromatographic separations or by reaction of an appropriate functional group. For example reactions that produce colored product are measured in the visible region of the spectrum. Other reactions increase conjugation to permit measurement in the UV region.

IR analysis is primarily used for identification of decomposition products and has found very few quantitative applications in stability evaluations. Nuclear magnetic resonance is finding increasing number of applications since it offer sspecificity along with simplicity of operation. Mass spectrometry in combination with GC or HPLC has been found useful in some cases for monitoring impurities in pharmaceutical compounds.

3. Selecting the detector:

The detector should determine the sample components within atleast a 1000 fold concentration range from 100% to 0.1-0.05% of the parent drug.

DRUG PROFILE

CIPROFLOXACIN HYDROCHLORIDE (AHFS drug information, 2004)

Structure:

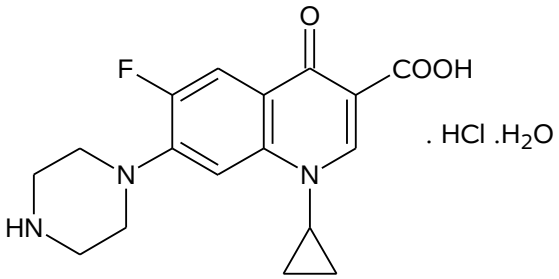


Figure-1 Structure of Ciprofloxacin hydrochloride

MECHANISM OF ACTION :

Ciprofloxacin is bactericidal and acts by inhibiting the A sub unit of DNA gyrase (topoisomerase) which is essential in the reproduction of bacterial DNA.

PHARMACOKINETICS :

Ciprofloxacin is rapidly and well absorbed from gastro intestinal tract. Its bioavailability is about 70%, Protein binding ranges from 20-40% and plasma

half-life is about 3.5 hours. It is widely distributed in the body and tissue penetration is generally good. It is eliminated by urine excretion but non renal clearance may account for about a third of elimination

DRUG INTERACTION :

Administration of ciprofloxacin with theophylline may lead to elevated serum concentration of theophylline and prolongation of its elimination half-life.

Ciprofloxacin have also been shown to interfere with the metabolism of caffeine. Concurrent administration of ciprofloxacin with antacids containing magnesium, aluminium, or calcium with sucralfate or divalent or trivalent cat ions such as iron may substantially interfere with the absorption of ciprofloxacin resulting in serum and urine levels considerably lower than desired.

DOSAGE :

Adult oral dose 250-750mg twice daily depending on the severity and nature of the infection. Intravenous dose is 100-400mg twice daily. Single oral dose of 750mg is suggested for surgical infection prophylaxis, given 60-90 minutes before the procedure.

METRONIDAZOLE (AHFS drug information, 2004)

Structure:

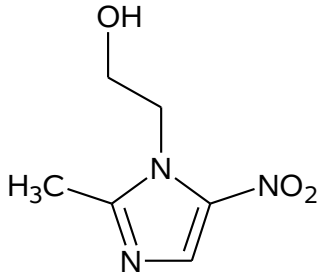


Figure-2 Structure of Metronidazole

ADMINISTRATION AND DOSAGE:

It is commonly used orally. Occasionally it is used intra vaginally in hospitalized patients. It is used mainly as IV bolus along with other antibiotics.

Table: 1

Metronidazole dosage guidelines:

Infection	Route	Unit dose	Frequency	Duration	Daily dose
Anaerobic infections (lower respiratory tract and endocardium)	IV	15mg/kg	6 hrs	7-10 days	< 4 g
Prophylaxis (hepatic and renal diseases)	IV	15mg/kg	30-60 mins	Before 6 hrs	-

COMPATIBILY STUDIES :

Metronidazole is compatible with several cephalosporins in that cefamandole nafate, cefazolin sodium, cefatoxime sodium, ceftazidime, cefriaxone. It is also compatible with ciprofloxacin and gentamicin. It is incompatible with amino acids.

STABILITY :

Reconstituted IV solutions are stable for 6 hrs, when stored below 26ºC in room temperature. Use diluted and neutralized IV solutions within 24 hrs. Store ready to use solution at 15ºC to 30ºC, protected from light. Once further diluted in infusion solution and neutralized, the solution should be used before 24 hrs. Ready to use metronidazole products indicate that these may be refrigerated but that crystals may form. The crystal redissolves on warming to room temperature. Prolonged exposure to light will cause a darkening of product. Direct sunlight should be avoided.

CLINICAL USES :

- It is used in the treatment of genital infections with T.vaginalis in both female and males in high percentage of cases.
- It is an effective amoebicide and has become the agent of choice for treatment of all symptomatic forms of amoebicide.
- Metronidazole has become the drug of choice for the treatment of giardiasis.
- It is extremely used for the treatment of serious infections due to susceptible anaerobic bacteria including *Bacteriodes, Clostridium, etc.*

- **Falagas ME. et.al, (2007)** studied the use of fluoroquinolones for the treatment of intra-abdominal surgical infections and reported that, in six prospective non-randomized clinical studies of patients with intra-abdominal infections, the clinical success achieved with the use of fluoroquinolones ranged from 77% to 94%. They concluded fluoroquinolones to be an effective and relatively safe option for the treatment of patients with intra-abdominal infections.

- **Motwani SK. et,al, (2007)** validated a stability-indicating HPTLC method for the densitometric determination of moxifloxacin using precoated silica gel 60 F_{254} as stationary phase, n- propanol – ethanol – 6M ammonia solution (4:1:2, v/v/v) as mobile phase and detection at 298nm. Stability studies were performed by acid and alkali hydrolysis, oxidation, dry heat, wet heat treatment and photo degradation. As the method could effectively separate the drug from its degradation products, it could be employed as a stability-indicating method.

- **Fernandez-Varon E. et.al, (2006)** studied the stability of moxifloxacin injection in peritoneal dialysis solution bags (Dianeal PD1 1.36% and Dianeal PD1 3.86% were two glucose concentrations) by HPLC method and reported that the mean moxifloxacin concentration in the Dianeal PD1 1.36% solution remained ≥ 90% of the initial concentration for 14 days at 4 °C, 7 days at 25 °C and 3 days at 37 °C. For Dianeal PD1 3·86% moxifloxacin concentrations remained ≥ 90% for 14 days at 4 °C, 3 days at 25 °C and 12 h at 37 °C.

- **Matthaiou DK. et.al, (2006)** studied the comparative clinical trials of ciprofloxacin/metronidazole versus broad-spectrum β-lactam based regimens in the treatment of intra-abdominal infections and concluded that the ciprofloxacin/metronidazole combination may be superior to β-lactam-based

therapeutic regimens in the treatment of intra-abdominal infections with regard to cure of infections.

- **Jan Krzek. et.al, (2005)** developed a HPTLC method with densitometric detection for identification and quantification of ciprofloxacin, by using pre-coated silica gel 60 F_{254} as stationary phase and chloroform-methanol-25% ammonia (43+43+14 ,v/v/v) as mobile phase. The degraded products of Ciprofloxacin in pharmaceutical preparations were identified by different R_f values.

- **Werk R. et.al, (2005)** studied the effect of combination of ciprofloxacin with metronidazole for the treatment of mixed aerobic/anaerobic infections and concluded that metronidazole in combination with ciprofloxacin was slightly more potent against the tested *Clostridia* than ciprofloxacin or metronidazole alone.

- **Madan AK. et.al, (2004)** studied the use of ciprofloxacin in the treatment of hospitalized patients with intra-abdominal infections and after clinical trials they concluded that the combination of ciprofloxacin plus metronidazole was an effective and safe regimen for the treatment of intra-abdominal infections.

- **Voges M. et.al, (2004)** studied the stability of gentamicin, tobramycin, netilmicin, vancomycin, cefazolin, unfractionated heparin and low molecular weight heparin when added to four different peritoneal dialysis solutions (PD) solutions (Extraneal, Physioneal, Nutrineal, and Dianeal) in new, non PVC flex-containers. The study was carried out at two different temperatures (25°C and 37°C).

- **Yeung SM. et.al, (2004)** studied the pharmacokinetics of oral Ciprofloxacin in continuous cycling peritoneal dialysis (CCPD) patients. The concentration of Ciprofloxacin in blood samples was analyzed by HPLC method and also by microbiological assay. They finally concluded that oral administration of 750mg Ciprofloxacin in every 12 hours for CCPD patients

might be useful for empirical gram-negative coverage of CCPD peritonitis and for and for treatment of documented peritonitis caused by *E.coli* or *Klebsiella* species.

- **Vega E. et.al, (2001)** studied the compatability of ciprofloxacin-metronidazole admixture by first-derivative spectrophotometry using the zero-crossing technique of measurement and studied the effect of light on the admixture and concluded that the admixture was proved to be photolabile.

- **Vega E. et.al, (1999)** validated a reverse-phase LC method for quantitative analysis of intravenous admixtures of ciprofloxacin and metronidazole.

- **Bailie GR. et.al, (1995)** studied the stability of drug additives to peritoneal dialysate and reported that most beta-lactams were stable for 1-2 weeks in a refrigerator and for several days at room temperature. Aminoglycosides were stable for 1-2 days at room temperature. Glycopeptides were stable for several weeks in refrigerator or at room temperature. Prolonged storage at room temperature resulted in instability of cefotaxime, ceftazidime, cefriaxone and miconazole. They also added that additives should be made as close as possible to the time of the exchange or additives should be stored refrigerated, then warmed prior to use.

- **Kane MP. et.al, (1994)** studied the stability of ciprofloxacin injection in peritoneal dialysis solutions using high-performance liquid chromatography at 4,25 and 37°C and concluded that in peritoneal dialysis solutions containing 1.5% and 4.25% dextrose, ciprofloxacin remained stable for 2 weeks at 4°C,for 1 week at 25°C and for 2 days at 37°C.

- **Kelly A et al (2005)** studied the stability and compatibility of levofloxacin and metronidazole during simultaneous and actual Y-site administration and reported that admixtures of levofloxacin ready to use infusion solution (5mg/ml) were visually and chemically compatible at

approximately 23ºC for up to3 hours during simulated and actual infusion through a Y-site.

- **Mawhinney WM. et.al, (1992)** studied the stability of ciprofloxacin in peritoneal dialysis solution at 4, 20 and 37 degrees C. Samples withdrawn at different time intervals were analysed by high performance liquid chromatography and also by microbiological assay using *Pseudomonas aeruginosa*. The net percentage of change in ciprofloxacin concentration was 0.76% after storage at 4 degree C, 1.02% after storage at 20 degree C and 0.75% after storage at 37 degree C.

- **Goodwin SD. et.al, (1991)** studied the compatability of ciprofloxacin injection with selected drugs and solutions by high performance liquid chromatography and concluded that ciprofloxacin injection was compatible with gentamicin, metronidazole, and tobramycin and incompatible with aminophylline and clindamycin. The compatability of ciprofloxcacin- amikacin admixtures depended on the intravenous solution and storage temperature.

- **McCormick. et.al, (1987)** studied the effect of peritoneal dialysis fluid and pH on bactericidal activity of ciprofloxacin and concluded that the bactericidal activity of ciprofloxacin is not affected by pH or medium (peritoneal dialysis fluid).

EXPERIMENTAL WORK

INSTRUMENTS AND APPARATUS :

UV Spectrophotometer	**: Jasco V530**
HPTLC	: Camag
Refrigerator	: Kelvinator
B.O.D Incubator	: Technico
Incubator	***: Hybrid***
Hot air oven	: Chemie
Autoclave	: Kailash
Horizontal laminar air flow	: Serwell instruments
Analytical Balance	: Dhona, 200d
Non absorbent cotton	: Ramaraju surgicals
Inoculating loop	: Hi-media
Pipette (1,5 & 10ml)	: Borosil.
Test tubes	***: Borosil***
Standard Flask (10, 100ml)	: Borosil
Sterile swabs	***: Hi-media***
HPTLC plates	: Merck
Petri dishes	: Borosil
Micropipettes (20-200µl)	: Ebrapipette
Micropipettes (50-100µl)	: Varipipette
Microtips	: Tarson
Cultural tubes (20ml)	: Borosilicate type-2 Borosil
Mueller Hinton Agar media	: Hi-media.

CHEMICALS USED:

Ciprofloxacin injection (2mg/ml)	: Cipla
Metronidazole injection (0.5% v/v)	: Nirlife Health Care.
Water for injection	: Core Health Care.
Peritoneal dialysis fluid	: Parenteral Drugs (India) Ltd
Chloroform	: Qualigens Fine Chemicals
Methanol	: Qualigens Fine Chemicals
25% Ammonia	: S D Fine Chem. Limited

METHODOLOGY:

CALIBRATION GRAPH OF CIPROFLOXACIN I.V USING FIRST DERIVATIVE UV SPECTROPHOTOMETRIC METHOD: (E.Vega. et.al, 2001)

a) PREPARATION OF STOCK SOLUTION:

From 2mg/ml of Ciprofloxacin I.V infusion 5 ml was taken and was made up to 100ml with sterile water to get a concentration of 100 µg/ml of Ciprofloxacin.

b) PROCEDURE:

From the above solution 0.2, 0.4, 0.6, 0.8 and 1.0 ml was pipetted and made upto10ml with sterile water to get concentrations of 2, 4, 6, 8 and 10 µg/ml respectively. The UV spectra and first derivative spectra of the above samples were taken and absorbance was measured at 263 nm.

PROTOCOL FOR THE STABILITY STUDY OF CIPROFLOXACIN I.V AT THREE DIFFERENT TEMPERATURES:

This study was carried out to determine the stability of Ciprofloxacin I.V at different temperature conditions of storage, i.e. refrigeration (5ºC), room temperature (25ºC) and at 45ºC. The parameters evaluated were changes in physical stability i.e. changes in pH, clarity and color. The chemical stability of ciprofloxacin was determined using stability indicating first derivative UV spectrophotometric method for quantification.

PROCEDURE:

2mg/ml Ciprofloxacin I.V infusion bottles were marked as refrigeration (5ºC), room temperature (25ºC) and 45ºC for identification and were kept at different storage conditions.

From the above samples serial dilutions were made with sterile water to get 8 µg/ml concentrations and absorbance was measured at 263nm. The above procedure was repeated for 5 days with samples withdrawn from solutions kept at 5ºC, 25ºC and 45ºC at various time intervals of 0min, 1hrs, 2hrs, 4hrs, 6hrs, 24hrs, 72hrs, and 120hrs respectively. The pH and clarity were also noted. The results obtained were observed and recorded.

CALIBRATION GRAPH OF METRONIDAZOLE I.V USING FIRST DERIVATIVE UV SPECTROPHOTOMETRIC METHOD: (E.Vega. et.al, 2001)

a) PREPARATION OF STOCK SOLUTION:

From 5mg/ml of Metronidazole I.V infusion 2ml was taken and was made up to 100 ml with sterile water to get a concentration of 100 µg/ml of Metronidazole.

b) PROCEDURE:

From the above solution 0.2, 0.4, 0.6, 0.8, 1.0 ml was pipetted and was made upto10ml with sterile water to get concentrations of 2, 4, 6, 8 and 10 µg/ml respectively. The UV spectra and first derivative spectra of the above samples were taken and the absorbance was measured at 299 nm.

PROTOCOL FOR THE STABILITY STUDY OF METRONIDAZOLE I.V AT THREE DIFFERENT TEMPERATURES:

This study was carried out to determine the stability of Metronidazole I.V at different temperature conditions of storage, i.e. refrigeration (5ºC), 25ºC and at 45ºC. The parameters evaluated were changes in physical stability i.e. changes in pH, clarity and color. The chemical stability of Metronidazole was determined using stability indicating first derivative UV spectrophotometric method for quantification.

PROCEDURE:

5mg/ml of Metronidazole infusions were marked as refrigeration (5ºC), room temperature (25ºC) and 45ºC for identification and were kept at different storage conditions.

From the above samples serial dilution was done with sterile water to get 8µg/ml concentration and absorbance was measured at 299nm. The above procedure was repeated for 5 days with samples withdrawn from vials kept at 5ºC, 25ºC and 45ºC at various time intervals of 0min, 1hrs, 2hrs, 4hrs, 6hrs, 24hrs, 72hrs, and 120hrs respectively. The pH and clarity were also noted. The results obtained were observed and recorded.

CALIBRATION GRAPH OF CIPROFLOXACIN-METRONIDAZOLE I.V ADMIXTURE (BY SIMULTANEOUS UV SPECTROPHOTOMETRIC METHOD):

The ratio of 1: 2.5 (Ciprofloxacin: Metronidazole) was used, because the same ratio was used during admixture of two drugs in clinical practice. Five different concentration mixtures of same ratio were prepared (1: 2.5, 2: 5, 3: 7.5, 4: 10, 5: 12.5) and used for calibration graph.

PROCEDURE:

From the 2mg/ml of Ciprofloxacin I.V infusion and 5mg/ml of Metronidazole infusion aliquot dilutions were made in the ratio of 1: 2.5 to get 1, 2, 3, 4 and 5µg/ml concentration of Ciprofloxacin and 2.5, 5.0, 7.5, 10 and 12.5µg/ml concentration of Metronidazole respectively.

The UV spectra and first derivative spectra of the above solutions were taken and the absorbance was measured at 263 nm for Ciprofloxacin and 299 nm for Metronidazole and calibration graph were plotted.

PROTOCOL FOR STABILITY STUDY OF CIPROFLOXACIN-METRONIDAZOLE I.V ADMIXTURE AT THREE DIFFERENT TEMPERATURES (BY SIMULTANEOUS UV SPECTROPHOTOMETRIC METHOD):

This study was carried out to determine the stability of Ciprofloxacin-Metronidazole I.V admixture at different temperature conditions of storage, i.e. refrigeration (5ºC), 25ºC and at 45ºC. The parameters evaluated were changes in physical stability i.e. changes in pH, clarity and color. The chemical stability of ciprofloxacin and metronidazole were determined from the calibnration graph prepared earlier.

2mg/ml of Ciprofloxacin I.V infusion and 5mg/ml of Metronidazole I.V infusion solutions were mixed in the ratio of 1: 2.5 and were marked as refrigeration (5ºC), room temperature (25ºC) and 45ºC for identification and were kept at different storage conditions.

PROCEDURE:

From the samples at different temperatures aliquot dilutions were made to get a concentration of 4 µg/ml of Ciprofloxacin and 10 µg/ml of Metronidazole (ratio 1: 2.5). The UV spectra and first derivative spectra of the solution were taken and the absorbance was measured at 263nm for Ciprofloxacin and at 299 nm for Metronidazole respectively. The above procedure was repeated for 5 days with samples withdrawn from vials kept at 5ºC, 25ºC and 45ºC at various time intervals of 0min, 1hrs, 2hrs, 4hrs, 6hrs, 24hrs, 72hrs, and 120hrs respectively. The parameters evaluated were changes in physical stability i.e. changes in pH and clarity were also noted. The results obtained were observed and recorded.

CALIBRATION GRAPH FOR CIPROFLOXACIN I.V IN PERITONEAL DIALYSIS SOLUTION USING HPTLC METHOD: (Jan Krzek. et.al, 2005)

a) PREPARATION OF STOCK SOLUTION :

From 2mg/ml of Ciprofloxacin I.V infusion 2 ml was taken and was made up to 10 ml with peritoneal dialysis solution to get a concentration of 400µg /ml. From this solution 1 ml was taken and made up to 10 ml with peritoneal solution to give 40µg/ml.

b) PROCEDURE :

From the above drug solution 0.5µl, 1.0µl, 1.5µl, 2.0µl, 2.5µl with corresponding concentrations of 0.02, 0.04, 0.06, 0.08 and 0.1 µg/spot were spotted in HPTLC plates of size 10 × 10 cm. The spots were dried and developed using chloroform: methanol: 25% ammonia (4.3:4.3:1.4) as solvent system. Peak areas were measured at 277nm.

HPTLC CONDITIONS :

Stationary phase	: Silica gel $60F_{254}$
Mobile phase	: Chloroform: methanol: 25% ammonia (4.3: 4.3:1.4)
UV detector	: 277nm
Injector	: Linomat injector
Scanner	: CAMAG Scanner
Method	: Ascending chromatography
Calibration range	: 0.02 to 0.1µg/spot

PROTOCOL FOR STABILITY STUDY OF CIPROFLOXACIN I.V IN PERITONEAL DIALYSIS SOLUTION AT THREE DIFFERENT TEMPERATURES (Quantification by HPTLC method)

This study was carried out to determine the stability of Ciprofloxacin in peritoneal dialysis solution at different temperature conditions of storage, i.e. refrigeration (5ºC), 25ºC and at 45ºC. The parameters evaluated were changes in physical stability i.e. changes in pH, clarity and color. The chemical stability of Ciprofloxacin was determined from the calibration graph prepared earlier.

From 2mg/ml of Ciprofloxacin I.V infusion 2ml was taken and made up to 10ml with peritoneal dialysis solution to get a concentration of 400µg /ml and were marked as 45ºC, 25ºC and 5ºC for identification and kept at different conditions for stability studies. The study was performed in duplicate.

b) PROCEDURE:

From the above solution serial dilutions were made to get 40µg/ml. From this solution 0.5µl, 1.0µl, 1.5µl, 2.0µl, 2.5µl of drug solution was spotted corresponding to concentrations of 0.02, 0.04, 0.06, 0.08, 0.1 µg/spot in HPTLC plates of size 10 × 10 cm and developed in a solvent system (Jan Krzek. et.al, 2005) comprising of Chloroform: methanol: 25% ammonia (4.3: 4.3: 1.4), dried and peak areas were measured at 277nm. The above procedure was repeated for 5 days with samples withdrawn from vials kept at 5ºC, 25ºC and 45ºC at various time intervals of 0min, 1hrs, 2hrs, 4hrs, 6hrs, 24hrs, 72hrs, and 120hrs respectively. The pH and clarity were also noted. The results obtained were observed and recorded.

CALIBRATION GRAPH OF CIPROFLOXACIN I.V IN PERITONEAL DIALYSIS SOLUTION BY MICROBIOLOGICAL ASSAY (K.B. Method) USING *E.COLI* – NCIM 2911 AS TEST ORGANISM :

a) PREPARATION OF STOCK SOLUTION :

From 2mg/ml of Ciprofloxacin I.V infusion aliquots of 1, 2, 3, 4,and 5 ml were taken and made up to 10 ml with peritoneal dialysissolution to get 200, 400, 600, 800, 1000µg /ml concentration respectively.

b) PROCEDURE :

From each of the above solutions, 10µl was added to sterile disc to get concentration of 2, 4, 6, 8, and 10µg /disc respectively. The sterile discs were placed on Muller Hinton agar plates, which were previously swabbed by using *E.coli* –NCIM 2911as test organism. The plates were incubated for 24 hours at 37ºC and observed for zone of inhibition. The blank peritoneal dialysis solution containing disc was also kept and confirmed for no inhibitory action (zone of inhibition) against the test micro organism.

PROTOCOL FOR STABILITY STUDY OF CIPROFLOXACIN I.V IN PERITONEAL DIALYSIS SOLUTION BY MICROBIOLOGICAL ASSAY (K.B. Method) USING *E.COLI* – NCIM 2911 AS TEST ORGANISM :

From 2mg/ml of Ciprofloxacin I.V infusion 2ml was taken and made up to 10ml with peritoneal dialysis solution to get a concentration of 400µg /ml and were marked as 5ºC, 25ºC and 45ºC for identification and kept at different storage conditions for stability studies. The study was performed in duplicate.

PROCEDURE :

From the above solution 10µl was pipetted out to contain 4µg of Ciprofloxacin and was added to the sterile disc kept on Muller Hinton agar plates, which were previously swabbed by using *E.coli* as test organism. The plates were incubated for 24 hours at 37ºC and observed for zone of inhibition. The above procedure was repeated at various time intervals of sampling.

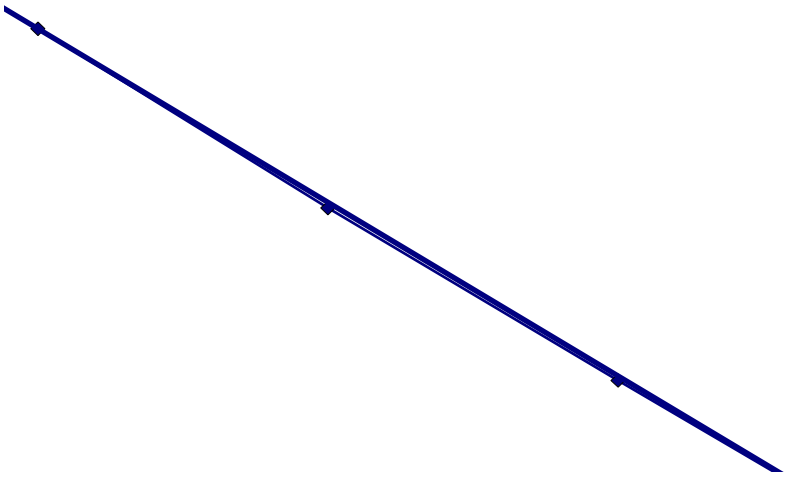
RESULTS AND DISCUSSION

Table: 2

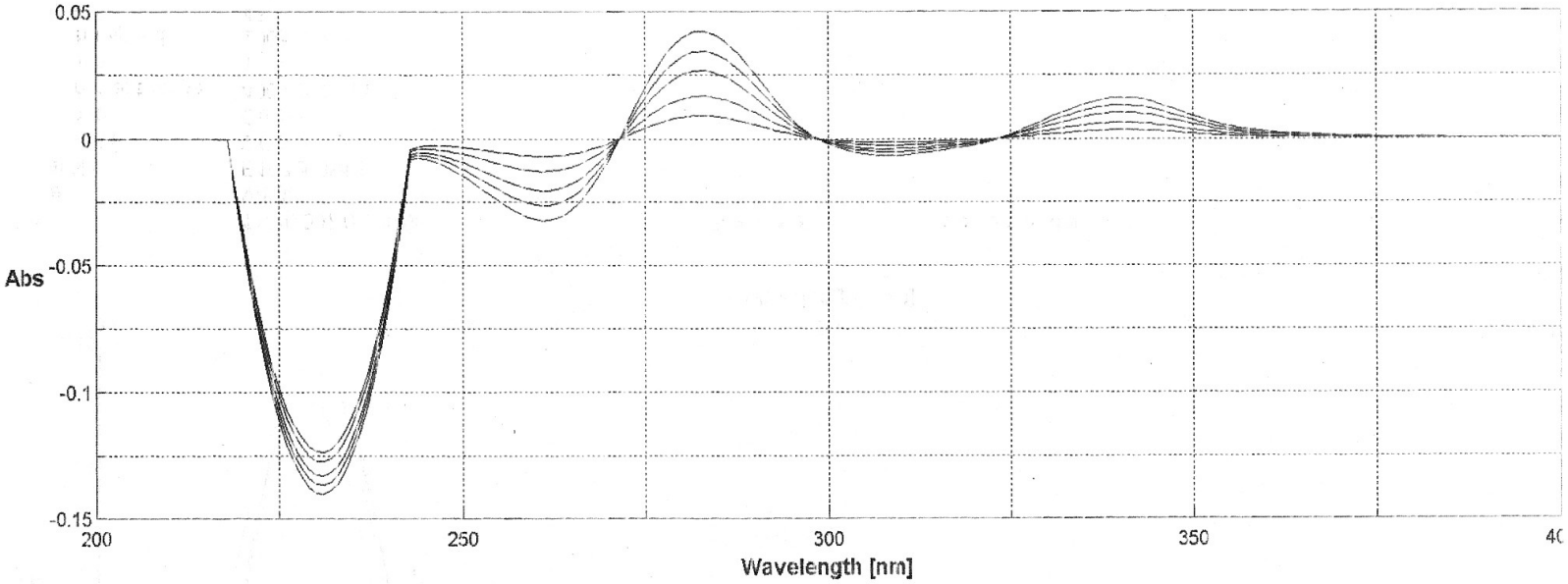
CALIBRATION GRAPH OF CIPROFLOXACIN HYDROCHLORIDE I.V USING FIRST DERIVATIVE SPECTROPHOTOMETRIC METHOD:

CONCENTRATIONS IN µg/ml	*ABSORBANCE AT 263nm*
2 µg/ml	-0.00675
4 µg/ml	-0.01251
6µg/ml	-0.01995
8µg/ml	-0.02564
10 µg/ml	-0.03139

Figure: 3



Calibration graph of Ciprofloxacin IV using first derivative spectrophotometric method



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Sample name			
Operator	College of Pharmacy		
Comment			

Table: 3

PHYSICAL STABILITY OF CIPROFLOXACIN HYDROCHLORIDE I.V AT THREE DIFFERENT TEMPERATURES:

Temperature		**0 hour**	**1 hour**	**2 hour**	**4 hour**	**6 hour**	**24 hour**	**72 hour**	**120 hour**
5ºC	**pH**	4.1	4.1	4.1	4.1	4.1	3.9	3.9	3.8
	Clarity	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear
	Color	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless
25ºC	**pH**	4.1	4.1	4.1	4.1	4.0	3.9	3.8	3.7
	Clarity	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear
	Color	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless
45ºC	**pH**	4.1	4.1	4.0	3.9	3.9	3.8	-	-
	Clarity	Clear	Clear	Clear	Clear	Clear	Clear	-	-
	Color	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	-	-

Table: 4

CHEMICAL STABILITY OF CIPROFLOXACIN HYDROCHLORIDE I.VAT THREE DIFFERENT TEMPERATURES:

(Values given below are average of two samplings)

Temp	Expected Conc.	0 hour	1 hour	2 hour	4 hour	6 hour	24 hour	72 hour	120 hour
5ºC	8µg/ml	-0.02560 (7.99)	-0.02534 (7.91)	-0.02514 (7.85)	-0.02494 (7.79)	-0.02455 (7.67)	-0.02403 (7.51)	-0.02319 (7.25)	-0.02274 (7.11)
25ºC	8µg/ml	-0.02560 (7.99)	-0.02531 (7.90)	-0.02495 (7.79)	-0.02447 (7.64)	-0.02390 (7.46)	-0.02303 (7.19)	-0.02271 (7.09)	-0.02188 (6.83)
45ºC	8 µg/ml	-0.02560 (7.99)	-0.02482 (7.75)	-0.02433 (7.60)	-0.02294 (7.17)	-0.02194 (6.86)	0.01858 (5.81)	-	-

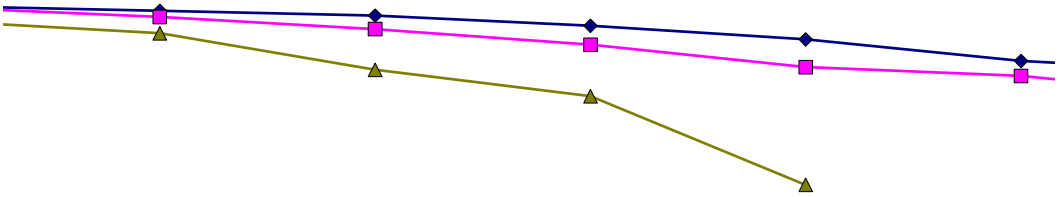
Values recorded are absorbance values and those given in brackets are concentration of ciprofloxacin in µg/ml. Values in bold represents the 10% degradation from the initial concentration.

Table: 5

DEGRADATION PROFILE OF CIPROFLOXACIN HYDROCHLORIDE I.VAT THREE DIFFERENT TEMPERATURES:

Sampling Time	Amount of Drug Remaining (%)		
	5ºC	25ºC	45ºC
0 time	100	100	100
1 hour	99.1	98.9	96.9
2 hour	98.4	97.5	95.1
4 hour	97.7	95.7	89.7
6hour	96.2	93.4	85.8
24 hour	94.2	90.1	72.7
72 hour	91.0	88.8	-
120 hour	89.3	85.6	-

Figure: 4



STABILITY STUDY OF CIPROFLOXACIN HYDROCHLORIDE I.V AT THREE DIFFERENT TEMPERATURE CONDITIONS (BY FIRST DERIVATIVE SPECTROPHOTOMETRIC METHOD) (E.Vega. et.al, 2001)

Ciprofloxacin hydrochloride I.V (2mg/ml) was stored at different temperatures such as room temperature (25ºC), refrigeration (5ºC) and 45ºC. Samples were withdrawn at different time intervals for 5 days and quantified by UV first derivative spectrophotometric method. The observations at the end of 5 days were noted.

The stability of ciprofloxacin hydrochloride i.v stored at refrigeration temperature proved to be more stable even after 72 hours (% deviation = 9.0) than the other solutions stored at room temperature (% deviation = 11.2) and 45ºC (% deviation = 27.3).

At 45ºC, the % deviation was > 10% (considered unstable) after nearly 2 hours of storage (value indicates between 2-4 hours), at 25ºC > 10% degradation was between 24 hours and 72 hours and at 5ºC drug solution was seen between 72 hours and 120 hours.

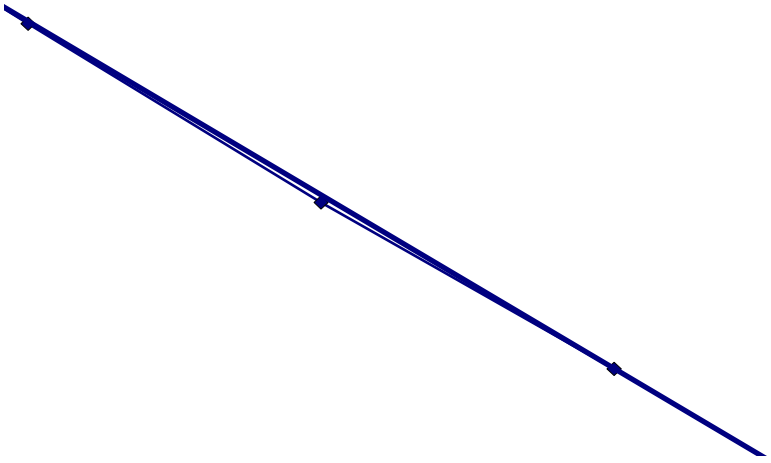
The results obtained from pH, color and clarity test showed physical stability at 5ºC even after 5 days.

Table: 6

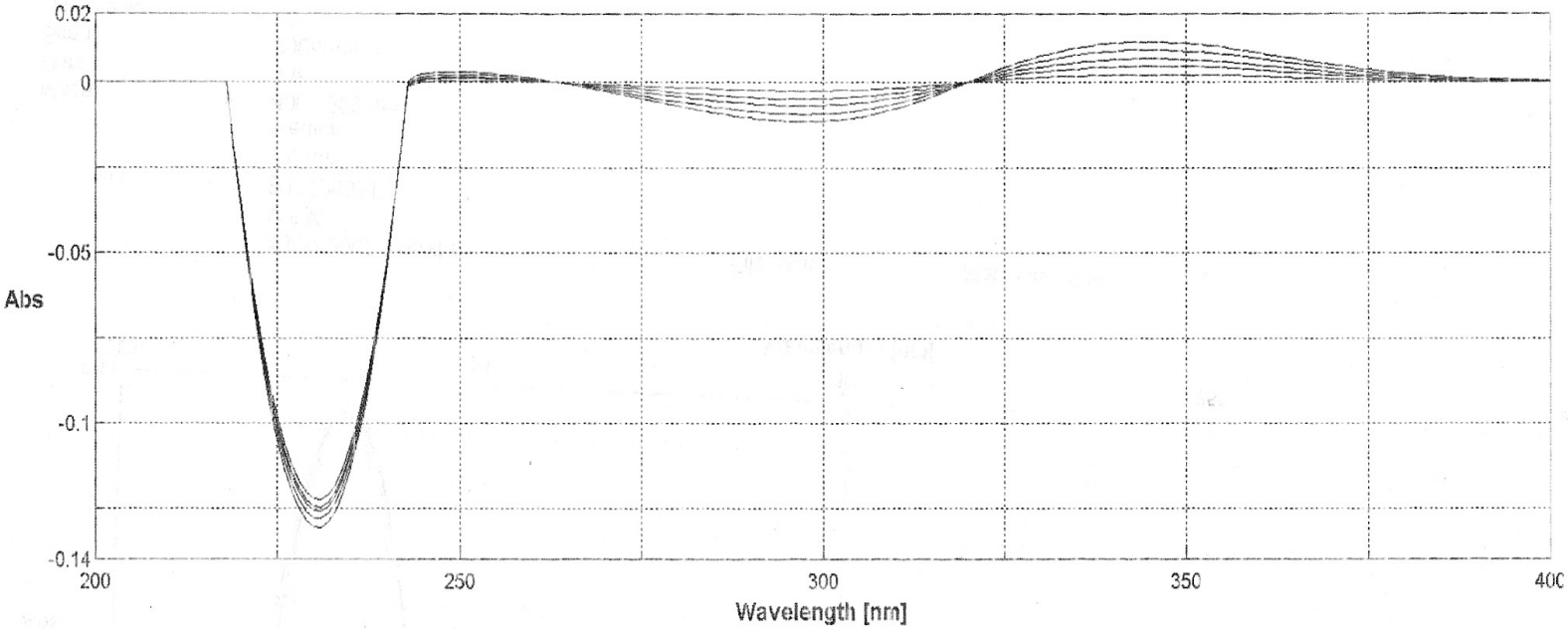
CALIBRATION GRAPH OF METRONIDAZOLE I.V USING FIRST DERIVATIVE SPECTROPHOTOMETRIC METHOD:

CONCENTRATIONS IN µg/ml	*ABSORBANCE AT 299nm*
2 µg/ml	-0.00239
4 µg/ml	-0.00465
6 µg/ml	-0.00667
8 µg/ml	-0.00916
10 µg/ml	-0.01135

Figure: 5



Calibration graph of Metronidazole IV using first derivative spectrophotometric method



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No. of cycle	1		
Sample name			
Operator	College of Pharmacy		

Table: 7

PHYSICAL STABILITY OF METRONIDAZOLE I.V. AT THREE DIFFERENT TEMPERATURES:

Temperature		**0 hour**	**1 hour**	**2 hour**	**4 hour**	**6 hour**	**24 hour**	**72 hour**	**120 hour**
5ºC	**pH**	4.9	4.9	4.9	4.8	4.8	4.7	4.6	4.5
	Clarity	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear
	Color	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless
25ºC	**pH**	4.9	4.9	4.9	4.9	4.8	4.7	4.6	4.6
	Clarity	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear
	Color	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless
45ºC	**pH**	4.9	4.9	4.8	4.6	4.5	4.4	-	-
	Clarity	Clear	Clear	Clear	Clear	Clear	Clear	-	-
	Color	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	-	-

Table: 8

CHEMICAL STABILITY OF METRONIDAZOLE I.V. AT THREE DIFFERENT TEMPERATURES:

(Values given below are average of two samplings)

Temp	Expected Conc.	0 hour	1 hour	2 hour	4 hour	6 hour	24 hour	72 hour	120 hour
5ºC	8µg/ml	-0.00916 (7.98)	-0.00902 (7.89)	-0.00897 (7.83)	-0.00884 (7.77)	-0.00876 (7.71)	-0.00866 (7.65)	-0.00831 (7.47)	-0.00790 (7.19)
25ºC	8µg/ml	-0.00916 (7.98)	-0.00900 (7.87)	-0.00891 (7.82)	-0.00878 (7.70)	-0.00866 (7.60)	-0.00818 (7.18)	-0.00790 (6.99)	-0.00740 (6.49)
45ºC	8µg/ml	-0.00916 (7.98)	-0.00897 (7.83)	-0.00883 (7.71)	-0.00829 (7.24)	-0.00815 (7.12)	-0.00641 (5.60)	-	-

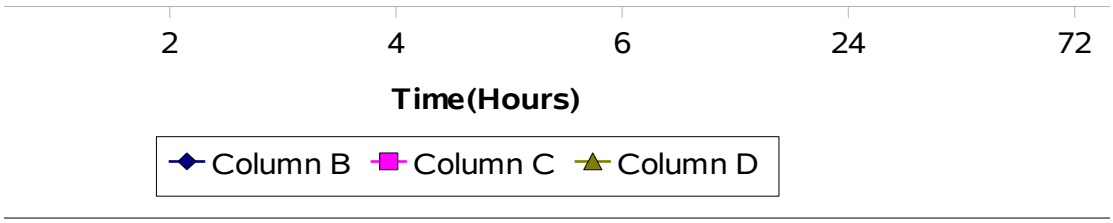
Values recorded are absorbance values and those given in brackets are concentration of metronidazole I.V. in µg/ml. Values in bold represents the 10% degradation from the initial concentration.

Table: 9

DEGRADATION PROFILE OF METRONIDAZOLE AT THREE DIFFERENT TEMPERATURES:

Sampling Time	Amount of Drug Remaining (%)		
	5ºC	25ºC	45ºC
0 time	100	100	100
1 hour	99.3	99.0	98.4
2 hour	98.6	98.4	96.9
4 hour	97.9	96.9	91.0
6hour	97.2	95.7	89.5
24 hour	96.5	90.5	70.5
72 hour	94.3	88.2	-
120 hour	90.8	81.9	-

Figure: 6



STABILITY STUDY OF METRONIDAZOLE AT THREE DIFFERENT TEMPERATURE CONDITIONS (BY FIRST DERIVATIVE SPECTROPHOTOMETRIC METHOD) (E.Vega. et.al, 2001)

Metronidazole (5mg/ml) was stored at different temperatures such as room temperature (25ºC), refrigeration (5ºC) and 45ºC. Samples were withdrawn at different time intervals for 5 days and quantified by UV first derivative spectrophotometric method. The observations at the end of 5 days were noted.

The stability of metronidazole stored at refrigeration temperature proved to be more stable even after 120 hours (% deviation = 9.2) than the other solutions stored at room temperature (% deviation = 18.1) and 45ºC (% deviation = 29.5).

At 45ºC, the % deviation was > 10% (considered unstable) after nearly 4 hours of storage (value indicates between 4-6 hours), at 25ºC > 10% degradation was between 24 hours and 72 hours and at 5ºC drug solution was stable even after 5 days of sampling.

The results obtained from pH, color and clarity test showed physical stability at 5ºC even after 5 days.

Table: 10

CALIBRATION GRAPH FOR SIMULTANEOUS ESTIMATION:

Concentration of ciprofloxacin is 1, 2, 3, 4 and 5µg/ml and that of metronidazole is 2.5, 5.0, 7.5, 10.0 and 12.5µg/ml.

RATIO OF CIPROFLOXACIN: METRONIDAZOLE	CONCENTRTION OF CIPROFLOXACIN AT 263 nm	CONCENTRATION OF METRONIDAZOLE AT 299 nm
1:2.5	-0.00328	-0.00286
2:5	-0.00627	-0.00611
3:7.5	-0.01004	-0.00855
4:10	-0.01301	-0.01118
5:12.5	-0.01766	-0.01352

Figure: 7

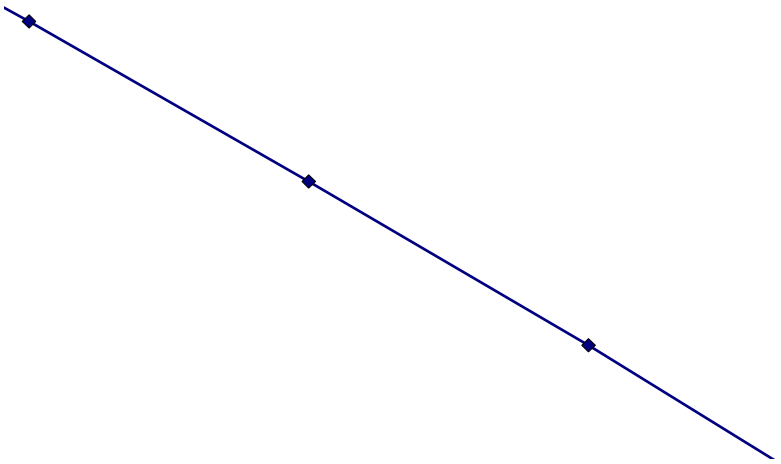
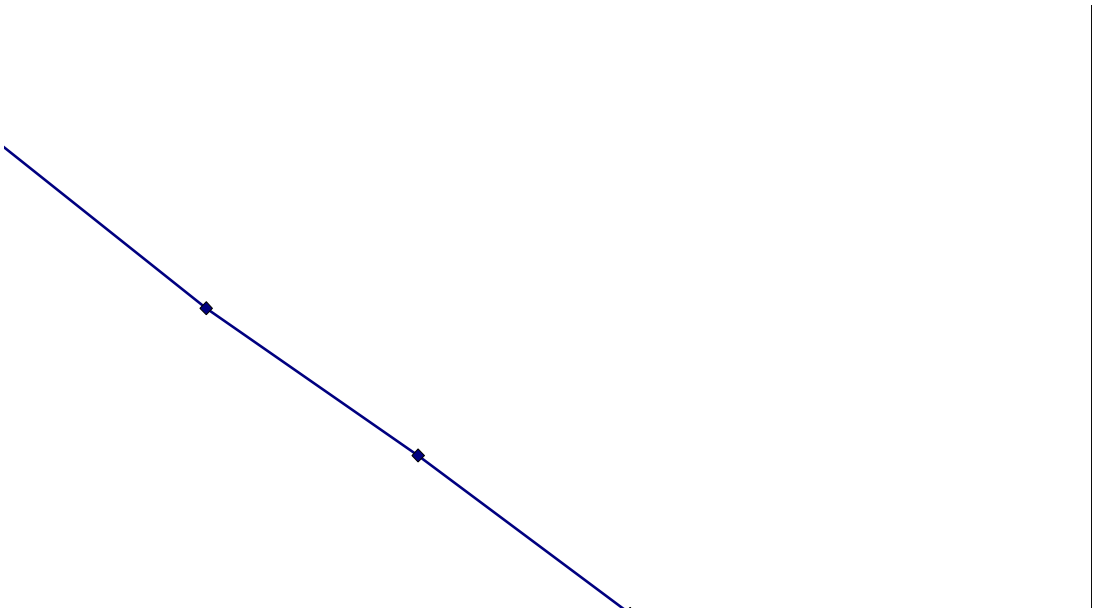
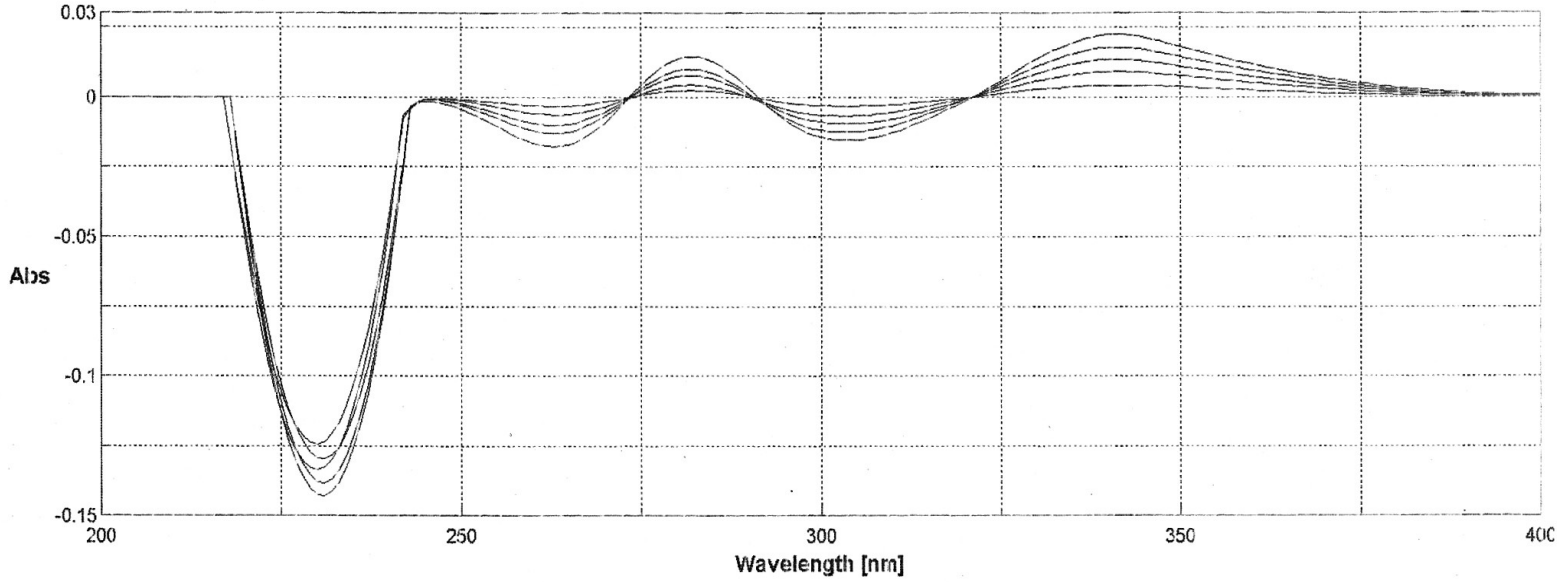


Figure: 8



Calibration graph for Simultaneous Estimation using first derivative spectrophotometric method



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Operator	College of Pharmacy		
Comment			

Table: 11

PHYSICAL STABILITY OF CIPROFLOXACIN-METRONIDAZOLE I.V ADMIXTURE AT THREE DIFFERENT TEMPERATURES:

Temperature		**0 hour**	**1 hour**	**2 hour**	**4 hour**	**6 hour**	**24 hour**	**72 hour**	**120 hour**
5ºC	**pH**	4.2	4.2	4.2	4.2	4.2	4.1	4.0	3.9
	Clarity	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear
	Color	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless
25ºC	**pH**	4.2	4.2	4.1	4.1	4.0	4.0	3.9	3.8
	Clarity	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear
	Color	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless
45ºC	**pH**	4.2	4.2	4.2	4.1	4.1	4.0	-	-
	Clarity	Clear	Clear	Clear	Clear	Clear	Clear	-	-
	Color	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	-	-

Table: 12

CHEMICAL STABILITY OF CIPROFLOXACIN-METRONIDAZOLE ADMIXTURE I.VAT THREE DIFFERENT TEMPERATURES: (Values given below are average of two samplings)

Temp	Expected Conc.	0 hour	1 hour	2 hour	4 hour	6 hour	24 hour	72 hour	120 hour
5ºC	Ciprofloxacin 4µg/ml	-0.01300 (3.99)	-0.01286 (3.95)	-0.01272 (3.91)	-0.01249 (3.84)	-0.01222 (3.76)	-0.01186 (3.65)	-0.01166 (3.59)	-0.01130 (3.48)
	Metronidazole 10µg/ml	-0.01112 (9.94)	-0.01105 (9.88)	-0.01083 (9.68)	-0.01071 (9.58)	-0.01063 (9.51)	-0.01039 (9.30)	-0.01021 (9.14)	-0.01000 (8.96)
25ºC	Ciprofloxacin 4µg/ml	-0.01300 (3.99)	-0.01277 (3.92)	-0.01254 (3.85)	-0.01244 (3.82)	-0.01212 (3.72)	-0.01169 (3.59)	-0.01117 (3.43)	-0.01097 (3.37)
	Metronidazole 10µg/ml	-0.01112 (9.94)	-0.01101 (9.85)	-0.01092 (9.77)	-0.01075 (9.61)	-0.01051 (9.40)	-0.01020 (9.12)	-0.00977 (8.74)	-0.00948 (8.48)
45ºC	Ciprofloxacin 4µg/ml	-0.01300 (3.99)	-0.01244 (3.82)	-0.01217 (3.74)	-0.01106 (3.40)	-0.01063 (3.27)	-0.00923 (2.84)	-	-
	Metronidazole 10µg/ml	-0.01112 (9.94)	-0.01087 (9.72)	-0.01031 (9.22)	-0.00938 (8.39)	-0.00914 (8.18)	-0.00811 (7.26)	-	-

Values recorded are absorbance values and those given in brackets are concentration of ciprofloxacin-metronidazole in µg/ml. Values in bold represents the 10% degradation from the initial concentration.

Table: 13

DEGRADATION PROFILE OF CIPROFLOXACIN-METRONIDAZOLE I.V ADMIXTURE AT THREE DIFFERENT TEMPERATURES:

Sampling Time	Amount of drug remaining (%)					
	5ºC		25ºC		45ºC	
	Ciprofloxacin	Metronidazole	Ciprofloxacin	Metronidazole	Ciprofloxacin	Metronidazole
0 time	100	100	100	100	100	100
1 hour	99.1	99.3	98.4	99.1	95.7	97.7
2 hour	98.2	97.3	96.7	98.3	93.7	92.7
4 hour	96.7	96.4	95.8	96.7	85.2	84.4
6 hour	94.9	95.8	93.5	94.6	81.9	82.3
24 hour	92.3	93.7	90.2	91.8	71.3	73.1
72 hour	90.8	92.1	86.1	88.4	-	-
120 hours	88.1	90.3	84.7	85.8	-	-

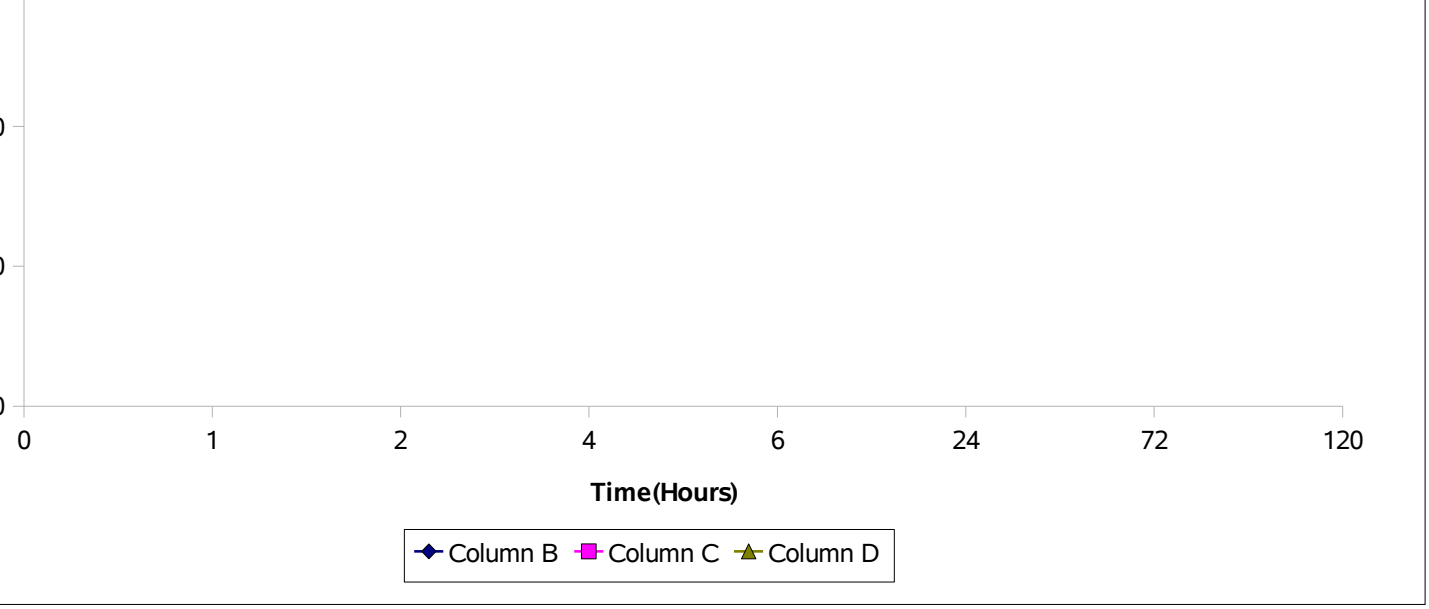


Figure: 10

STABILITY STUDY OF CIPROFLOXACIN-METRONIDAZOLE I.V ADMIXTURE AT THREE DIFFERENT TEMPERATURE CONDITIONS (BY FIRST DERIVATIVE SPECTROPHOTOMETRIC METHOD) (E.Vega. et.al, 2001)

Ciprofloxacin-metronidazole I.V admixture was stored at different temperatures such as room temperature (25ºC), refrigeration (5ºC) and 45ºC. Samples were withdrawn at different time intervals for 5 days and quantified by UV first derivative spectroscopic method. The observations at the end of 5 days were noted.

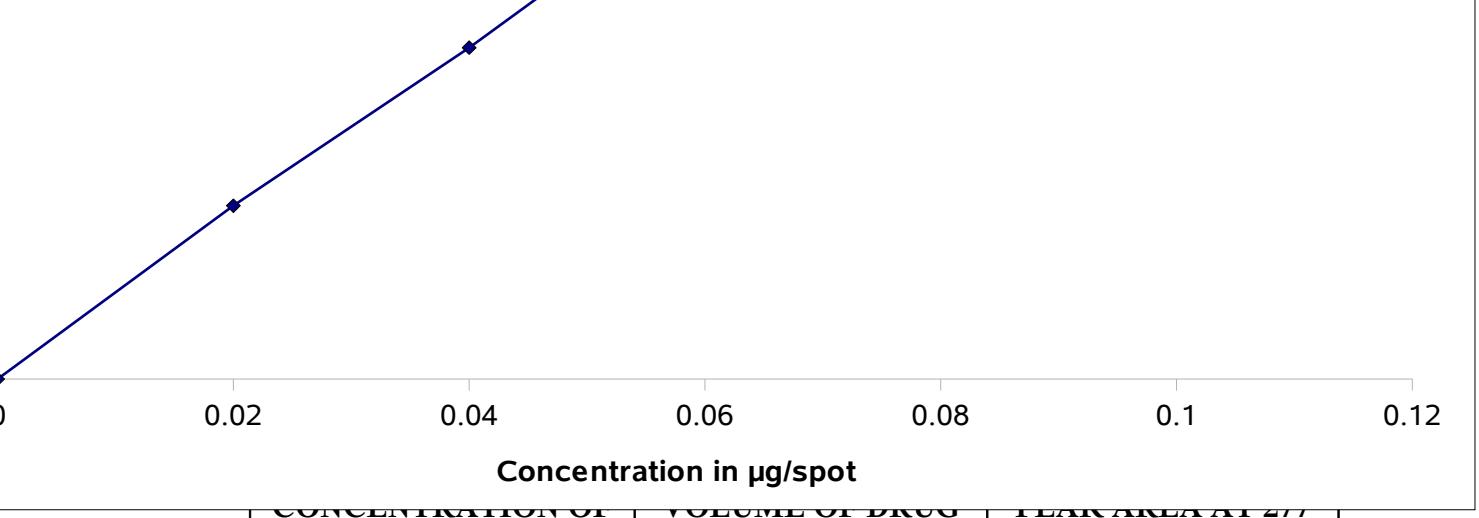
The stability of ciprofloxacin stored at refrigeration temperature proved to be more stable even after 72 hours (% deviation = 9.2) than the other solutions stored at room temperature (% deviation = 13.9) and 45ºC (% deviation = 28.7).

The stability of metronidazole stored at refrigeration temperature proved to be more stable even after 120 hours (% deviation = 9.7) than the other solutions stored at room temperature (% deviation = 14.2) and 45ºC (% deviation = 26.9).

At 45ºC, the % deviation of ciprofloxacin was > 10% (considered unstable) after nearly 2 hours of storage (value indicates between 2-4 hours), at 25ºC > 10% degradation was seen between 24 hours and 72 hours and at 5ºC drug solution was stable between 72 hours and 120 hours.

At 45ºC, the % deviation of metronidazole was > 10% (considered unstable) after nearly 2 hours of storage (value indicates between 2-4 hours), at 25ºC > 10% degradation was seen between 24 hours and 72 hours and at 5ºC drug solution was stable up to 5 days.

The results obtained from pH, color and clarity test showed physical stability at 5ºC even after 5 days.

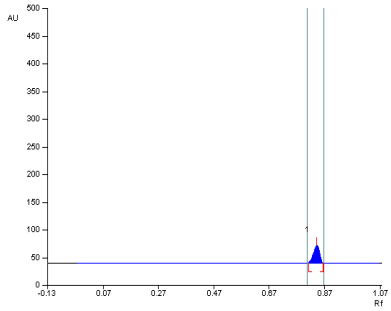


CONCENTRATION OF DRUG SPOTTED	VOLUME OF DRUG SPOTTED	PEAK AREA AT 277 nm
0.02µg/spot	0.5µl	650.3
0.04µg/spot	1.0µl	1243.5
0.06µg/spot	1.5µl	1888.0
0.08µg/spot	2.0µl	2524.1
0.10µg/spot	2.5µl	3061.7

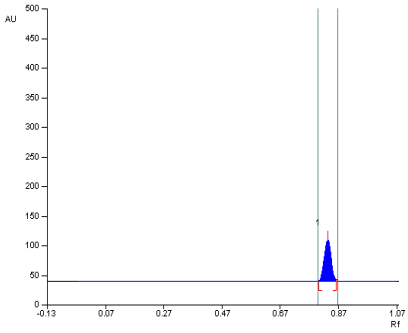
Figure: 11

HPTLC CHROMATOGRAMS OF CIPROFLOXACIN I.V IN PERITONEAL DIALYSIS SOLUTION

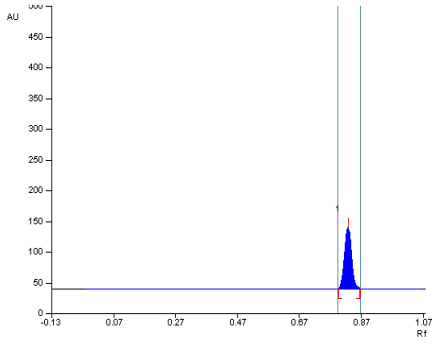
Standard graph



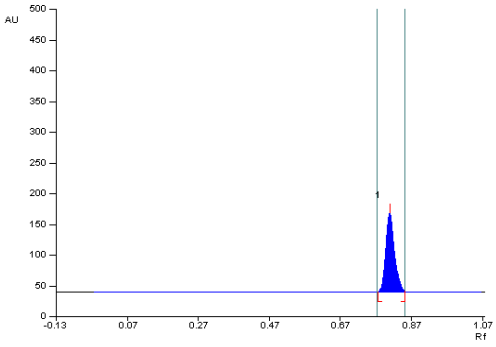
0.02mcg/spot



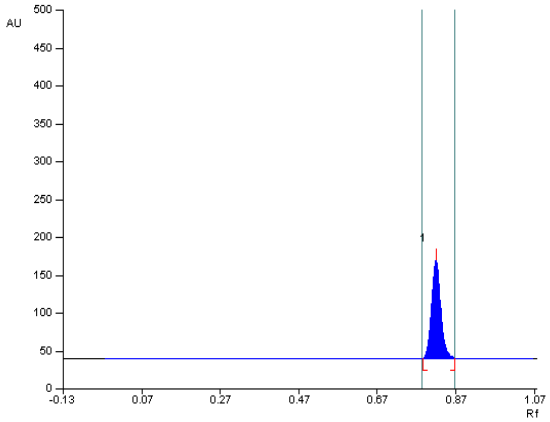
0.04mcg/spot



0.06mcg/spot



0.08mcg/spot



0.1mcg/spot

Table: 15

PHYSICAL STABILITY OF CIPROFLOXACIN I.V IN PERITONEAL DIALYSIS SOLUTION AT THREE DIFFERENT TEMPERATURES:

Temperature		**0 hour**	**1 hour**	**2 hour**	**4 hour**	**6 hour**	**24 hour**	**72 hour**	**120 hour**
5ºC	**pH**	4.6	4.6	4.6	4.6	4.6	4.4	4.4	4.4
	Clarity	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear
	Color	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless
25ºC	**pH**	4.6	4.6	4.6	4.6	4.5	4.4	4.4	4.4
	Clarity	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear
	Color	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless
45ºC	**Ph**	4.6	4.6	4.5	4.5	4.4	4.2	-	-
	Clarity	Clear	Clear	Clear	Clear	Clear	Clear	-	-
	Color	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	-	-

Table: 16

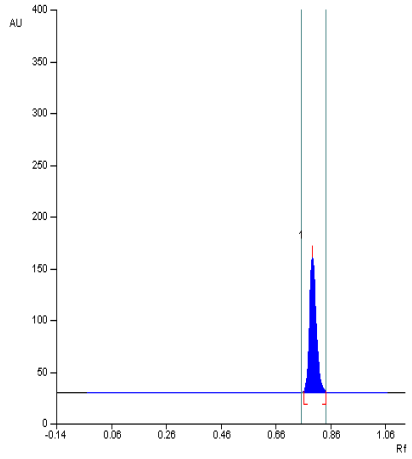
CHEMICAL STABILITY OF CIPROFLOXACIN I.V IN PERITONEAL DIALYSIS SOLUTION AT THREE DIFFERENT TEMPERATURES : (Values given below are average of two samplings)

Temp	Expected Conc.	0 hour	1 hour	2 hour	4 hour	6 hour	24 hour	72 hour	120 hour
5ºC	0.04µg/spot	1231.0 (0.039)	1224.8 (0.038)	1218.5 (0.038)	1212.5 (0.037)	1206.1 (0.037)	1181.1 (0.035)	1156.2 (0.034)	1118.9 (0.033)
25ºC	0.04µg/spot	1231.0 (0.039)	1221.1 (0.038)	1213.7 (0.037)	1198.9 (0.036)	1185.3 (0.035)	1152.0 (0.034)	1110.1 (0.032)	1042.4 (0.030)
45ºC	0.04µg/spot	1231.0 (0.039)	1206.1 (0.038)	1174.3 (0.037)	1110.8 (0.035)	1047.3 (0.033)	983.8 (0.031)	-	-

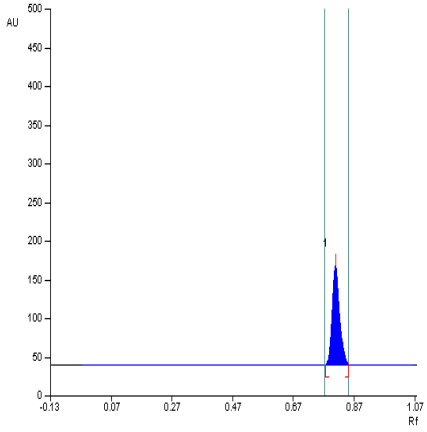
Values recorded are peak area values and those given in brackets are concentration of ciprofloxacin in peritoneal dialysis solution in mcg/spot. Values in bold represents the 10% degradation from the initial concentration.

Figure : 12

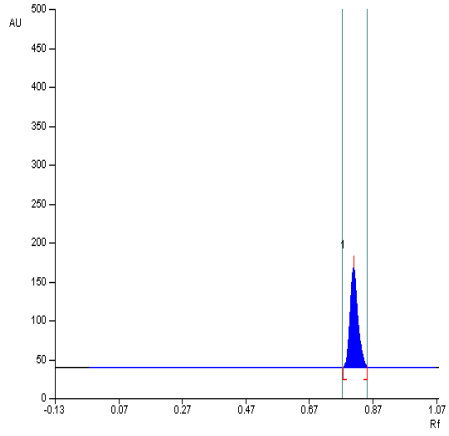
HPTLC CHROMATOGRAMS OF CIPROFLOXACIN I.V IN PERITONEAL DIALYSIS SOLUTION AT THREE DIFFERENT TEMPERATURES



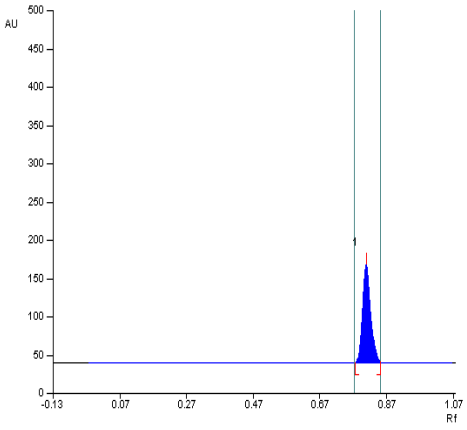
0 time



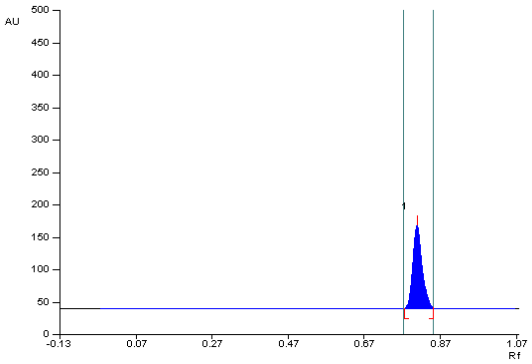
1 hour - 5ºC



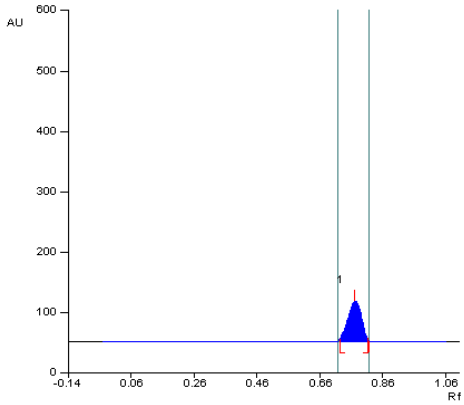
1 hour - 25ºC



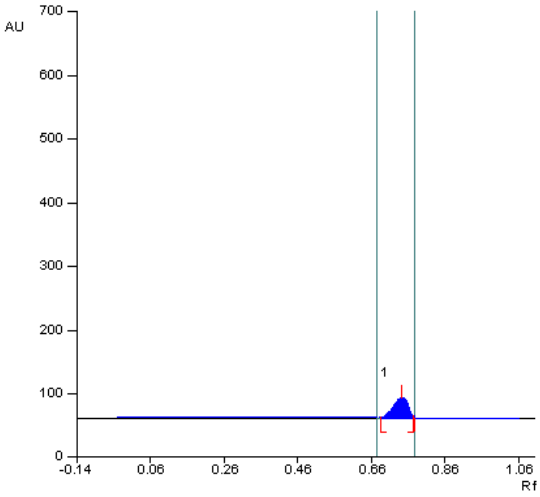
1 hour - 45ºC



6 hours - 5ºC



6 hours - 25ºC



6 hours - 45ºC

Table: 17

DEGRADATION PROFILE OF CIPROFLOXACIN IN PERITONEAL DIALYSIS SOLUTION AT THREE DIFFERENT TEMPERATURES:

Sampling Time	Amount of Drug Remaining (%)		
	5ºC	25ºC	45ºC
0 time	100	100	100
1 hour	99.5	97.4	97.4
2 hour	98.98	94.8	94.8
4 hour	98.50	92.2	89.6
6hour	97.97	89.6	84.4
24 hour	95.93	84.3	79.2
72 hour	93.90	79.3	-
120 hour	90.87	74.3	-

Figure: 13

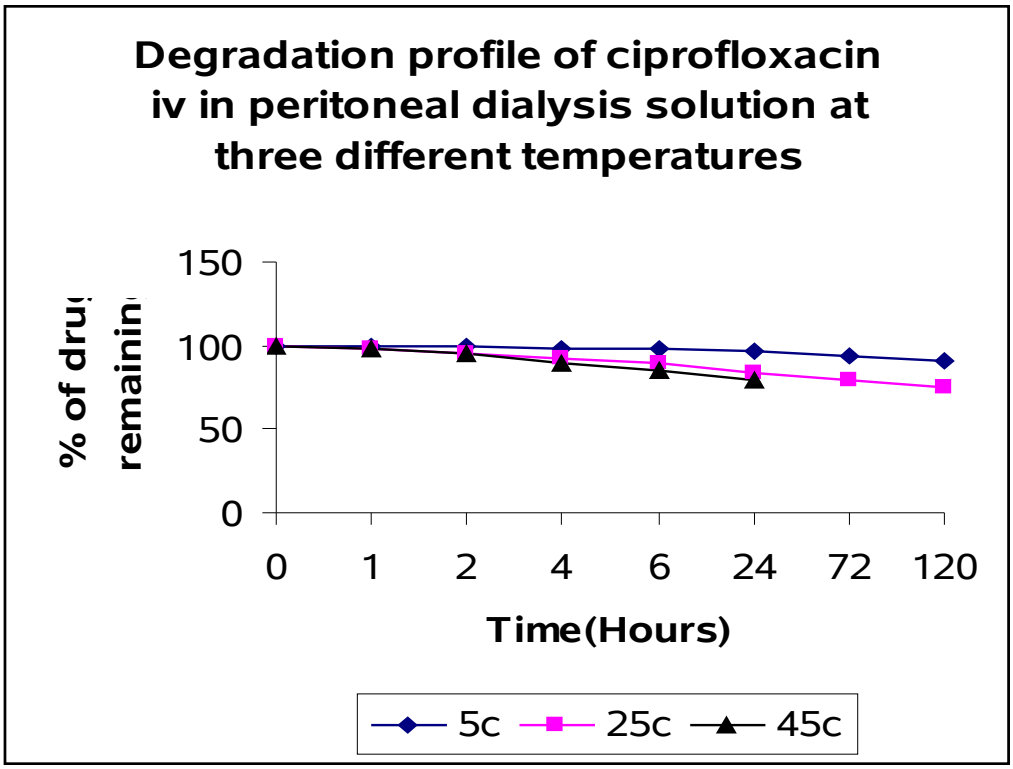


Table: 18

CALIBRATION GRAPH OF CIPROFLOXACIN I.V IN PERITONEAL DIALYSIS SOLUTION BY MICROBIAL ASSAY USING *E. COLI* – NCIM 2911 AS TEST ORGANISM:

S.No	Concentration (µg/disc)	Log Concentration	Average diameter of zone of inhibition (in mm) n=3
1.	2	0.3010	28.5
2.	4	0.6020	30.5
3.	6	0.7781	32
4.	8	0.9030	33.5
5.	10	1.0000	35.5

Figure: 14

ZONE OF INHIBITION OF CIPROFLOXACIN I.V IN PERITONEAL DIALYSIS SOLUTION BY MICROBIOLOGICAL ASSAY METHOD USING *E.COLI* – NCIM 2911 AS TEST ORGANISM

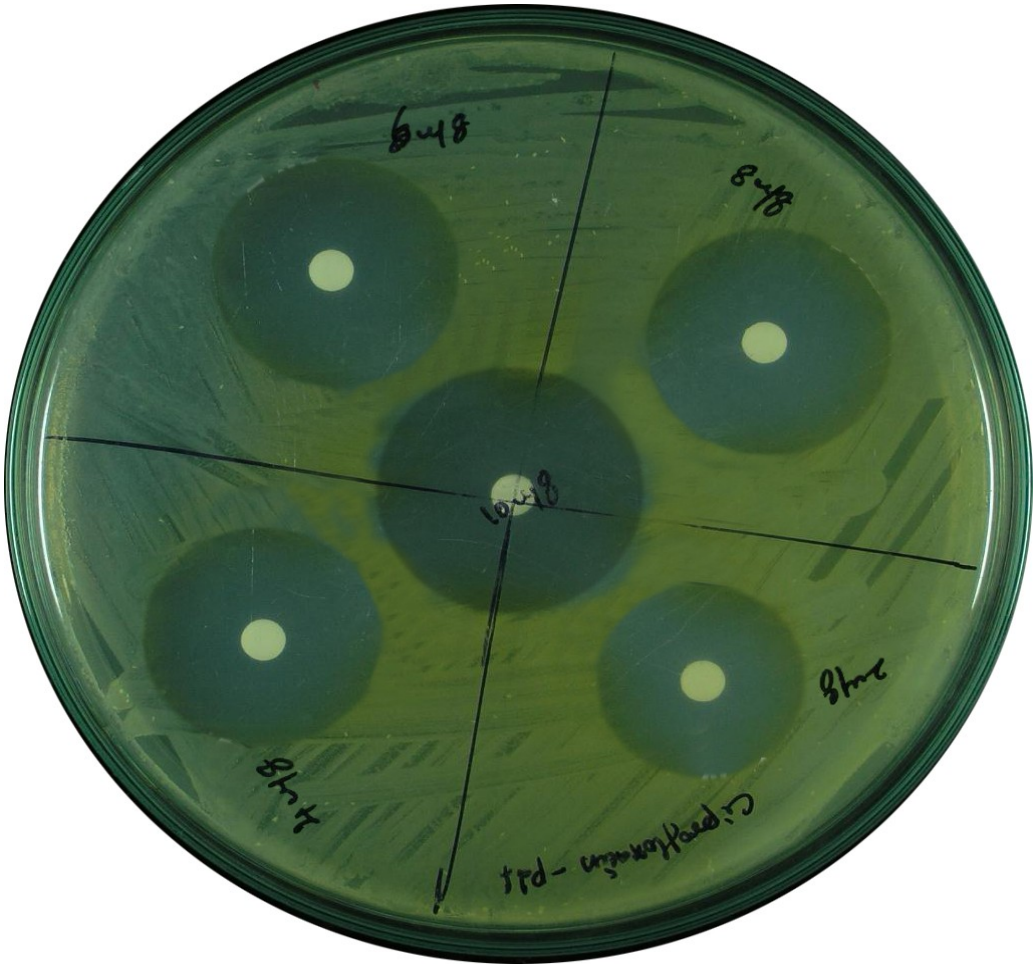
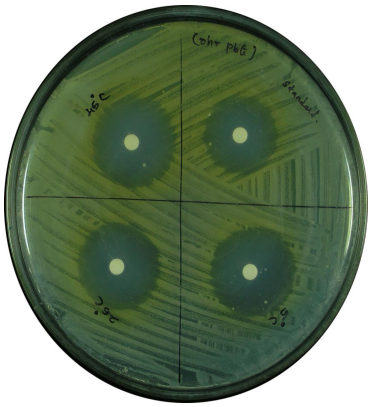


Figure : 15

ZONE OF INHIBITION OF CIPROFLOXACIN IN PERITONEAL DIALYSIS SOLUTION BY MICROBIOLOGICAL ASSAY METHOD USING *E.COLI* – NCIM 2911 AS TEST ORGANISM.

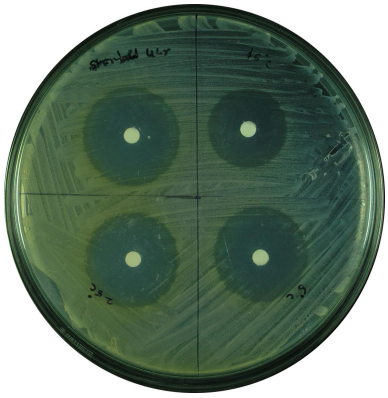
0 hour



1 hour

2 hour

4 hour



6 hour

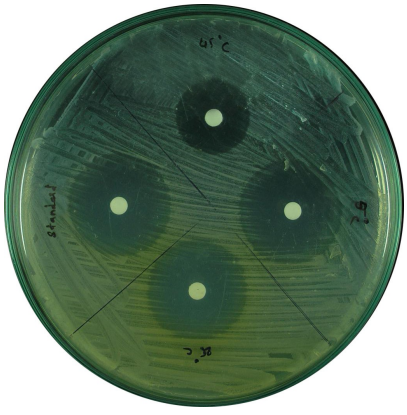


Table: 19

ZONE OF INHIBITION AND CONCENTRATIONS (WITH PERCENTAGE

DEVIATION) OF CIPROFLOXACIN I.V IN PERITONEAL DIALYSIS SOLUTION BY MICROBIAL ASSAY USING *E.COLI* – NCIM 2911 AS TEST ORGANISM:

Time of Sampling	Expected Concentration	Diameter of zone of inhibition		
		Refrigeration (5ºC)	Room Temp (25ºC)	(45ºC)
0	4µg/10µl	30	30	30
1 hr	4µg/10µl	30	30	30
2 hr	4µg/10µl	30	30	30
4 hr	4µg/10µl	30	30	29
6 hr	4µg/10µl	30	29	28

CONCLUSION OF WORK

Results of our study on the physical and chemical stability of Ciprofloxacin I.V alone (by stability indicating first derivative spectrophotometric method) found that Ciprofloxacin was stable for up to 5 days at refrigeration temperature, up to 24 hours at room temperature and for 4 hours at 45ºC.

Results of our study for Metronidazole I.V alone (by stability indicating first derivative spectrophotometry) found that Metronidazole was stable for up to 5 days at refrigeration temperature, up to 24 hours at room temperature and for 6 hours at 45ºC.

Results of our study for stability study of Ciprofloxacin-Metronidazole I.V admixture (by stability indicating first derivative spectrophotometry) were found similar to those reported by Vega.E et.al., 2001; where the admixture was stable up to 5 days at refrigeration temperature, 24 hours at room temperature, we inferred from our results that the admixture was stable for less than 4 hours at 45ºC.

Results of our study for stability of Ciprofloxacin I.V in peritoneal dialysis solution (by HPTLC method) were found similar to those reported by Kane MP et al 1994 and Mawhinney WM et al 1992; where Ciprofloxacin in peritoneal dialysis solution was stable for up to 5 days at refrigeration temperature and 24 hours at room temperature, we inferred from our results that Ciprofloxacin in peritoneal dialysis solution was stable only for 4 hours at 45ºC.

Results of our study involving Ciprofloxacin in peritoneal dialysis solution (quantified by microbiological assay method) that Ciprofloxacin in peritoneal dialysis solution was stable for only 4 hours at 45ºC. Good correlation was seen between the HPTLC assay and microbiological assay used in our work for the quantification of ciprofloxacin in peritoneal dialysis solution.

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